National Toxicology Program
U.S. Department of Health and Human Services

DRAFT NTP MONOGRAPH ON HEALTH EFFECTS OF LOW-LEVEL LEAD

OCTOBER 14, 2011

Office of Health Assessment and Translation
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Peer Review Date: November 17-18, 2011

TABLES OF CONTENTS

LIST OF TABLES AND FIGURES

TABLES:

FIGURES:

CONTRIBUTORS

Technical Advisors	
David Bellinger, Ph.D	Neuroepidemiology Unit, Children's Hospital, Harvard University, Boston, MA
David Lawrence, Ph.D	New York State Department of Health, Albany, NY
Barbara Materna, Ph.D, CIH	Chief, Occupational Health Branch, California Department of Public Health, Richmond, CA
Ana Navas-Acien, MD, Ph.D	Assistant Professor, Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Virginia Weaver, MD, MPH	Associate Professor, Department of Environmental Health Sciences, Division of Occupational and Environmental Health, Johns Hopkins Bloomberg School of Public Health, MD

NTP Staff

NIEHS/NTP Office of Health Assessment and Translation (OHAT)

Andrew A. Rooney, Ph.D (Project Lead)

Abee L. Boyles, Ph.D

Kyla Taylor, MS

Kembra L. Howdeshell, Ph.D

Vickie R. Walker, BS

Michael D. Shelby, Ph.D

Kristina A. Thayer, Ph.D (Director, OHAT)

NTP Office of Liaison, Policy and Review (OLPR)

Mary Wolfe, PhD (Director, OLPR and NTP Deputy Program Director for Policy)

Danica Andrews, BS

Denise Lasko

Lori White, Ph.D

NIOSH

Elizabeth A. Whelan, Ph.D. (Branch Chief, DHHS/CDC/NIOSH/DSHEFS/IWSB)

Science Writers	
Judy Stevens, BS	GLP Support Services (check location and affiliation)

ABBREVIATIONS

ABLES	Adult Blood Lead Epidemiology and Surveillance
ACCLPP	Advisory Committee on Childhood Lead Poisoning Prevention
ADHD	ADHD - attention deficit hyperactivity disorder
ALAD	aminolevulinate dehydratase
ALAD	δ-aminolevulinic acid dehydratase
ALS	Amyotrophic lateral sclerosis
AQCD	Air Quality Criteria Document
ATSDR	Agency for Toxic Substances and Disease Registry
BAEP	brainstem auditory evoked potentials
BASC	Behavioral Assessment System for Children
BP	blood pressure
BSI	Brief Symptom Inventory
CASAC	Clean Air Scientific Advisory Committee
CBC	Child Behavior Checklist
CTX	C-terminal telopeptides of type 1 collagen
DBP	DBP diastolic blood pressure
DRDB	self-reported delinquent behavior
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTH	delayed-type hypersensitivity
EBEs	early biological effect markers
ECG	Electrocardiographic
eGFR	estimated glomerular filtration rate
EPA	Environmental Protection Agency, US
ERG	electroreinographic
ET	Essential Tremor
FSIQ	Full-scale IQ
GCI	General Cognitive Index
HFE	hemochromatosis
IgE	Immunoglobulin e
IgG	immunoglobulin g
IgM	Immunoglobulin m
IUGR	intrauterine growth retardation
IVF	IVF in vitro fertilization
KTEA	Kaufman Test of Educational Achievement
MDI	Bayley Mental Developmental Index
MMSE	Mini-Mental State Examination
NAAQS	National Ambient Air Quality Standards
NAG	N-acetyl-β-D-glucosaminidase
NAS	Normative Aging Study
NHANES	National Health and Nutrition Examination Survey
NO	nitric oxide
NTP	National Toxicology Program

O^{2}-	superoxide
PINP	procollagen type-1 amino-terminal peptide
PIQ	performance IQ
PVC	polyvinyl chloride plastics
SATs	Standard Assessment Tests
SBP	systolic blood pressure
SCAN	screening test for auditory processing disorders
SD	Standard deviation
SDPTG-AI	second derivative finger photoplethysmogram waveform
SES	socioeconomic status
Th-1	helper T cell
VEP	visual evoked potentials
VIQ	verbal IQ
WHILA	Swedish Women's Health in the Lund Area
WISC	Wechsler Intelligence Scales for Children
WPPSI-R	Wechsler Preschool and Primary Scales of Intelligence
WRAT-Revised	Wide Range Achievement Test
ZPP	zinc protoporphyrin

1.0 EXECUTIVE SUMMARY

1.1 Introduction

Lead exposure remains a significant health concern despite policies and practices that have resulted in continued progress toward reducing exposure and lowering blood lead levels in the U.S. population. Lead (Pb) is one of the most extensively studied environmental toxicants, with more than 22,500 publications on health effects and exposure in the peer-reviewed literature.[1] While the toxicity associated with exposure to high levels of Pb was recognized by the ancient Greeks and Romans, the adverse health effects associated with low-level Pb exposure only became widely recognized in the second half of the 20th century. Over the past 40 years, epidemiological studies, particularly in children, continue to provide evidence of health effects at lower and lower blood Pb levels. In response, the Centers for Disease Control and Prevention (CDC) has repeatedly lowered the concentration defined as an elevated blood lead level in children (from 30μg/dL to 25μg/dL in 1985 and to the current level of 10μg/dL in 1991).

The purpose of this evaluation is to summarize the evidence in humans and reach conclusions whether health effects are associated with low-level Pb exposure as indicated by blood Pb levels <10μg/dL, with specific focus on the life stage associated with the health effect. The evaluation focuses on epidemiological evidence at blood lead levels <10μg/dL because health effects at higher blood lead levels are well established such that the definition for an elevated blood lead level is ≥10μg/dL for both children and adults (ABLES 2009; CDC 2010a). Pb was nominated (http://ntp.niehs.nih.gov/mtg?date=20100510&meeting=BSC) by the National Institute for Occupational Safety and Health for an NTP evaluation to assess the reproductive and developmental effects of Pb. The scope has been expanded from the original nomination to include an evaluation of health effects other than reproduction and development (e.g., cardiovascular effects in adults) in order to maximize the utility of the evaluation. Additional background information and updates until the NTP Monograph is finalized will be posted at http://ntp.niehs.nih.gov/go/36443.

1.2 Methods

The key questions and general approach for developing the conclusions on the health effects of low level Pb are outlined below. **Section 2.0** of this document contains additional details on the authoritative sources considered, the literature search strategy, and the peer review process.

1.2.1 Key Questions

What is the evidence that adverse health effects are associated with blood lead <10μg/dL?

- What reproductive, developmental, neurological, immune, cardiovascular, and renal health effect(s) are associated with blood lead levels <10μg/dL?
- What is the blood lead level associated with the health effect (i.e., <10μg/dL or <5μg/dL)?
- At which life stages (childhood or adulthood) is the effect identified?

[1] Based on a September 2011 PubMed search for keyword (MeSH) "lead" or "lead poisoning"

- ❖ Are there data to evaluate the association between bone Pb and the health effect and how does the association to this biomarker of Pb exposure compare to the association with blood Pb?

1.2.2 Approach to Develop Health Effects Conclusions

Conclusions in the NTP evaluation of Pb-related health effects in humans associated with low-level Pb were derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10µg/dL. The evaluation includes a review of the primary epidemiological literature for evidence that low-level Pb is associated with the following: reproductive and developmental effects, neurological effects, immunological effects, cardiovascular effects, and/or renal effects. These health effects were selected because there is a relatively large database of human studies in each area. The NTP considered four possible conclusions for specific health effects in each area:

> **Sufficient Evidence of an Association**: a relationship is observed between the exposure and health outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.
>
> **Limited Evidence of an Association**: an association is observed between the exposure and health outcome for which a causal interpretation is credible, but chance, bias, and confounding could not be ruled out with reasonable confidence.
>
> **Inadequate Evidence of an Association**: the available studies are insufficient in quality, consistency, or statistical power to permit a conclusion regarding the presence of absence of an association between exposure and health outcome, or no data in humans are available.
>
> **Evidence of No Association:** There are several adequate studies covering the full range of levels of exposure that humans are known to encounter (in this case limited to blood Pb levels <10µg/dL), which are mutually consistent in not showing an association between exposure to the agent and any studied endpoint.

The discussion of each health effect begins with a statement of the NTP's conclusion regarding whether the specific effect is associated with a blood Pb level <10µg/dL or <5µg/dL and the age group in which it is, or is not identified (childhood or adulthood) as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. For the purposes of this evaluation children refers to individuals <18 years of age unless otherwise specified. A lower effect level of 5µg/dL was selected because it is commonly used in epidemiological studies to dichotomize health-effects data by exposure levels and, therefore, data are often available to evaluate health effects for groups above and below this value. Key data and principal studies considered in developing the NTP's conclusions are then discussed in detail. Each section concludes with a summary discussing each health effect providing experimental animal data that pertain to the human evidence, and stating the basis for the NTP conclusions.

1.2.3 Appendices of Studies Considered

The information to support the NTP's conclusions for individual health effects is presented in each chapter. Human studies from populations with low-level Pb exposure that were considered in developing the conclusions are also abstracted for further reference and included in separate appendices for neurological effects, immune effects, cardiovascular effects, renal effects, and reproductive and developmental effects.

1.2.4 Authoritative Sources Considered

The NTP made extensive use of recent government evaluations of the health effects of Pb in the current assessment, especially the US Environmental Protection Agency (EPA) 2006 Air Quality Criteria Document (AQCD) for Lead (U.S. EPA 2006 and a draft updated version; 2011) which underwent extensive external public peer-review. In addition to the EPA 2006 AQCD for Lead, sources include the Agency for Toxic Substances and Disease Registry (ATSDR) 2007 Toxicological Profile for Lead (ATSDR 2007), and the CDC's Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) reports such as the 2010 Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women (CDC 2010b).

1.3 What does it mean to refer to blood Pb <10µg/dL?

The overwhelming majority of human epidemiological studies with individual Pb exposure data measured Pb in whole blood and this exposure metric serves as the basis for the evaluation of Pb levels <10µg/dL. An individual's blood Pb level reflects an equilibrium between current exogenous environmental Pb exposure and the endogenous body burden of Pb, primarily stored in bone (Factor-Litvak *et al.* 1999). Bone and teeth store 90-95% of the total body burden of Pb in adults, while bone Pb represents a smaller fraction (down to 70%) of the total body burden of Pb in young children (Barbosa *et al.* 2005; Barry 1981; Hu *et al.* 2007). The body eliminates Pb from circulating blood with a half-life of approximately one month, while bone is a more stable repository for Pb and therefore bone Pb levels reflect cumulative exposure to Pb integrated over years or even decades (Coon *et al.* 2006; Hu *et al.* 1998). The half-life of Pb in bone ranges from 10-30 years depending on the bone turnover rate, which in turn varies by type of bone and life stage (Rabinowitz 1991). In young children, continuous growth results in constant bone remodeling and bone Pb is exchanged with blood Pb much more frequently than in adults (Barbosa *et al.* 2005; Hu *et al.* 2007).

This evaluation focuses on the relationship between health effects and blood Pb levels because blood Pb is the most widely available exposure metric, blood Pb reflects the equilibrium between current and past exposure described above, and numerous studies have reported an association between blood Pb levels and health outcomes. However, measuring Pb in one tissue at one point in time does not present a complete picture of either current or cumulative Pb exposure, and bone Pb is superior to blood Pb in reflecting the long term stores of Pb in the body (Coon *et al.* 2006); therefore, bone Pb data were also considered when available. Note that measuring bone Pb is expensive, requires specialized equipment that is not generally accessible, and requires study subjects to travel to the location of the K-x-ray fluorescence apparatus.

Prior to bans on Pb in paint, solder, and gasoline, environmental Pb levels in the United States were higher, so older adults accumulated more Pb as children than children do today. Blood Pb levels in young children (ages 1-5 years) have decreased 10 fold over the last 30 years from a geometric mean of 15.1µg/dL in 1976-1980 to 1.51µg/dL in 2007-2008 (CDC 2007, 2011). While this is clearly good news for current populations of children, and represents a significant public health accomplishment, the majority of US children born before 1980 had blood Pb levels >10µg/dL during early childhood. Consequently, health effects in adults today may have been influenced by blood Pb levels >10µg/dL that many individuals experienced earlier in life.

Keeping childhood blood Pb levels in mind, there are data on multiple health effects in adults for which studies report a significant relationship between concurrent blood Pb levels as adults and the health effect (e.g., elevated blood pressure, reduced kidney function, or decreases in specific measures of cognitive function). There is a considerable body of evidence that these health effects are associated with Pb exposure and multiple studies report a significant association with concurrent blood Pb levels <10µg/dL. Furthermore the association with blood Pb is supported by the consistency of effects across epidemiological studies and biological coherence with animal data. It is well recognized that the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb without additional long term studies. Prospective studies in a population for which the data demonstrate that blood Pb levels remained consistently below 10µg/dL from birth until measurement of the outcome of interest would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL.

As described in **Section 1.2.2**, the NTP's conclusions were derived by evaluating data from epidemiological studies with a focus on blood Pb levels <10µg/dL. The evidence is discussed for specific health outcomes within each chapter, and varies by the study design and type of analysis used to examine the relationship of the health outcome with blood Pb across the hundreds of studies evaluated. In some cases, authors restricted the analysis to the population with blood Pb levels <10µg/dL, <5µg/dL, or even lower and the association of the health effect with the blood Pb level is clear. For example, Lanphear *et al.* (2000) reported an inverse relationship between blood Pb and academic performance in a cross-sectional study of 4853 children ages 6-16 from the NHANES-III dataset; the association with blood Pb remained significant in further analyses restricted to 4681 children with blood Pb <10µg/dL ($p<0.001$), and 4043 children with blood Pb <5µg/dL. In other cases, the authors reported a significant association between blood Pb and an effect in a population with a mean blood Pb level <10µg/dL (e.g., blood Pb level is associated with higher blood pressure in a study of 964 adults in the Baltimore Memory Study (Martin *et al.* 2006)). These analyses support an effect of a blood Pb level <10µg/dL, but they do not exclude the possibility that individuals significantly above or below the mean blood Pb level are driving the effect. Finally, in some studies, the authors compared effects between a Pb-exposed population and internal or external referents with lower blood Pb levels. For example, Naicker *et al.* (2010) compared the effect of a blood Pb level ≥5µg/dL with a blood Pb level <5µg/dL on developmental markers of puberty in a study of 682 girls age 13 in South Africa and found blood Pb ≥5µg/dL to be significantly associated with breast development, pubic hair development, and age of menarche.

1.4 Health Effects Evidence

1.4.1 NTP Conclusions

The NTP concludes that there is *sufficient* evidence for adverse health effects in children and adults at blood Pb levels below 10µg/dL and below 5µg/dL as well (see **Table 1.1** for summary of effect by life stage at which the effect is identified). A major strength of the evidence supporting effects of low-level Pb comes from the consistency demonstrated by adverse effects associated with blood Pb <10µg/dL across a wide range of health outcomes across major physiological systems from reproductive to renal, through multiple populations, from studies with substantial methodological heterogeneity, and for health outcomes in children and adults.

In children, there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with increased diagnosis of attention deficit hyperactivity disorder (ADHD), greater incidence of problem behaviors, and decreased cognitive performance as indicated by lower academic achievement and specific cognitive measures. There is also *limited* evidence that blood Pb <5µg/dL is associated with delayed puberty, decreased IQ, and decreased kidney function in children 12 and older. There is *sufficient* evidence that blood Pb levels <10µg/dL in children are associated with delayed puberty, reduced postnatal growth, and decreased cognitive performance as indicated by lower IQ. Although there is *sufficient* evidence that blood Pb levels of 10µg/dL and below are associated with elevated serum IgE, a principle mediatory of hypersensitivity, there is only *limited* evidence that blood Pb levels <10µg/dL are associated with changes to IgE-related health outcomes such as allergy diagnosed by skin prick test to common allergens. There is *inadequate* evidence of an association between blood Pb <10µg/dL in children and other allergic diseases such as eczema or asthma. There is also *inadequate* evidence of an association between blood Pb <10µg/dL and cardiovascular effects in children of any age, or renal function in children under age 12.

In adults, epidemiological data provide *sufficient* evidence that blood Pb levels <5µg/dL are associated with decreased renal function and blood Pb levels <10µg/dL are associated with increased blood pressure, hypertension, and increased cardiovascular-related mortality. There is *sufficient* evidence that maternal blood Pb levels <10µg/dL are associated with reduced fetal growth and *limited* evidence that they are associated with increased spontaneous abortion and preterm birth. The data also support a conclusion of *limited* evidence for an association between blood Pb <10µg/dL and decreased auditory function, neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and essential tremor, and decreases in specific measures of cognitive function in older adults. The NTP conclusions of an association between blood Pb levels <10µg/dL in adults and health effects cannot completely eliminate the potential contributing effects of early-life blood Pb levels as discussed in **Section 1.3**.

Although the relationship between many health effects and bone Pb as an exposure metric has not been examined, the data supports the importance of cumulative Pb exposure on cardiovascular effects of Pb in adults as well as neurocognitive decline in adults because the association between bone Pb and these endpoints is stronger than for blood Pb.

Table 1.1: NTP conclusions on health effects of low level Pb by life stage				
Life Stage	**Blood Pb Level**	**NTP Conclusion**	**Principal Health Effects**	**Bone Pb Evidence**
Children	<5µg/dL	*Sufficient*	Decreased academic achievement and specific cognitive measures, increased incidence of ADHD and problem behaviors	Tibia and dentin Pb are associated with ADHD, behavior, and cognition.
		Limited	Delayed puberty and decreased IQ, decreased kidney function in children age 12 years or older	The one available study of bone Pb in children does not support an association with postnatal growth.
	<10µg/dL	*Sufficient*	Delayed puberty, reduced postnatal growth, decreased IQ, decreased hearing, increased IgE*(not health outcome)	No data
		Limited	Increased hypersensitivity/allergy by skin prick test to allergens	No data
		Inadequate	Asthma, eczema, non-allergy immune function, cardiovascular effects, renal function children under age 12	No data
Adults	<5µg/dL	*Sufficient*	Decreased glomerular filtration rate	The one available study of bone Pb in the general population supports an association between bone Pb and decreased kidney function.
	<10µg/dL	*Sufficient*	Increased blood pressure, increased risk of hypertension, increased cardiovascular-related mortality; maternal blood Pb associated with reduced fetal growth	The association between bone Pb and cardiovascular effects is stronger than for blood. Maternal bone Pb is associated with reduced fetal growth.
		Limited	Psychological effects, decreased cognitive function, decreased hearing, increased incidence of ALS and essential tremor; maternal blood Pb associated with increased incidence of spontaneous abortion and preterm birth	The association between bone Pb and cognitive decline is stronger than for blood.
		Inadequate	Immune function, stillbirth, endocrine effects, birth defects, fertility or time to pregnancy**, and sperm parameters**	No data

Notes: ADHD - attention deficit hyperactivity disorder; IgE – immunoglobulin E; ALS - amyotrophic lateral sclerosis

*Increased serum IgE is associated with hypersensitivity; however, as described in **Section 1.4.3**, increased IgE does not equate to disease.

The NTP concludes that there is inadequate evidence that blood Pb levels <10µg/dL are associated with fertility, time to pregnancy, and sperm parameters; however, given the basis of the original nomination, the NTP evaluated the evidence that higher blood Pb levels (i.e., above 10µg/dL) are associated with reproductive and developmental effects and those conclusions are discussed in **Section 1.4.6 and presented in **Table 1.2**.

1.4.2 Neurological Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <10µg/dL are associated with adverse neurological effects in children and adults (see **Table 1.2** for summary of effects).

Unlike the dataset for most other health outcomes, there are a number of prospective studies of neurological effects that include prenatal exposure metrics (either maternal blood or cord blood). These prospective studies provide *limited* evidence that prenatal exposure to blood Pb levels <5µg/dL are associated with decreases in measures of general and specific cognitive function evaluated in children. There is also *limited* evidence that prenatal exposure to blood Pb levels <10µg/dL are associated with decreased IQ, increased incidence of ADHD and antisocial behavior problems, and decreased hearing measured in children. Conclusions on effects of prenatal exposure for outcomes evaluated as children are complicated by the high degree of correlation in childhood blood Pb levels over time and as described below, blood Pb levels during childhood are also associated with these outcomes.

In children, there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with broad based and specific indices of reduced cognitive function and an increase in ADHD diagnosis and antisocial behavioral problems. Lower levels of academic achievement determined by class rank and achievement tests have been reported in multiple prospective and cross-sectional studies of children with blood Pb <5µg/dL. A negative association between blood Pb at levels <5µg/dL and specific measures of cognitive function has been demonstrated in prospective and cross-sectional studies using a wide range of tests to assess cognitive function. Increased diagnosis of ADHD and behavioral problems are consistently reported in studies with mean blood Pb levels <5µg/dL. There is *sufficient* evidence that blood Pb levels <10µg/dL in children are associated with decreases in full-scale IQ (FSIQ) score and decreased auditory acuity. There is consistent evidence that blood Pb is associated with decreased IQ across multiple prospective studies of children and in well accepted pooled analyses (Lanphear *et al.* 2005) demonstrating effects at blood Pb levels <10µg/dL. Multiple cross-sectional studies reported hearing loss indicated by higher hearing thresholds and increased latency of brainstem auditory evoked potentials in children with blood Pb levels <10µg/dL.

In adults, there is *limited* evidence that blood Pb levels <10µg/dL are associated with psychiatric outcomes including anxiety and depression, decreased auditory function, neurodegenerative diseases including ALS and essential tremor, and decreases in specific measures of cognitive function in older adults. There are more consistent associations between bone Pb than blood Pb with decreases in cognitive function in adults, suggesting a role for cumulative Pb exposure.

1.4.3 Immune Effects

The NTP concludes that there is *limited* evidence that blood Pb levels <10µg/dL are associated with adverse immune effects in children and that there is *inadequate* evidence in adults (see **Table 1.2**).

Table 1.2: NTP conclusions on health effects of low level Pb by major health effect areas						
Health Area	**Population Or Exposure window**		**NTP Conclusion**	**Principal Health Effects**	**Blood Pb Evidence**	**Bone Pb Evidence**
Neurological	Prenatal		*Limited*	Decrease in measures of cognitive function	Yes, <5µg/dL	No data
			Limited	Decreased IQ, increased incidence of ADHD and problem behaviors, decreased hearing	Yes, <10µg/dL	No data
	Children		*Sufficient*	Decreased academic achievement and specific cognitive measures, increased incidence of ADHD and problem behaviors	Yes, <5µg/dL	Tibia and dentin Pb are associated with ADHD, behavior, and cognition.
			Sufficient	Decreased IQ, decreased hearing	Yes, <10µg/dL	No data
			Limited	Decreased IQ	Yes, <5µg/dL	No data
	Adults		*Limited*	Psychological effects, decreased hearing, decreased cognitive function, increased incidence of ALS and essential tremor	Yes, <10µg/dL	The association between bone Pb and cognitive decline is stronger than for blood.
Immune	Children		*Sufficient*	Increased IgE*(effect, not a health outcome)	Yes, <10µg/dL	No data
			Limited	Increased hypersensitivity/allergy by skin prick test to common allergens	Yes, <10µg/dL	No data
			Inadequate	Asthma, eczema	Unclear	No data
	Adults		*Inadequate*		Unclear	No data
Cardiovascular	Children		*Inadequate*		Unclear	No data
	Adults		*Sufficient*	Increased blood pressure, increased risk of hypertension, and increased cardiovascular-related mortality	Yes, <10µg/dL	The association between bone Pb and cardiovascular effects is stronger than for blood.
			Limited	Increased ECG abnormalities, heart rate variability	Yes, <10µg/dL	
Renal	Children under age 12		*Inadequate*		Unclear	No data
	Children 12 or older		*Limited*	Decreased glomerular filtration rate	Yes, <5µg/dL	No data
	Adults		*Sufficient*	Decreased glomerular filtration rate	Yes, <5µg/dL	Yes, one study
Reproductive and Developmental	Prenatal		*Limited*	Reduced postnatal growth	Yes, <10µg/dL	No data
	Children		*Sufficient*	Delayed puberty, reduced postnatal growth	Yes, <10µg/dL	One study does not support effects of bone Pb on growth.
			Limited	Delayed puberty	Yes, <5µg/dL	
	Adults	Women	*Sufficient*	Reduced fetal growth	Yes, <10µg/dL	Maternal tibia Pb is associated
			Limited	Increase in spontaneous abortion and preterm birth	Yes, <10µg/dL	No data
		Men	*Sufficient*	Adverse changes in sperm parameters and increased time to pregnancy	Yes, ≥15-20µg/dL	No data
			Limited	Decreased fertility	Yes, ≥10µg/dL	No data
			Limited	Increased spontaneous abortion	Yes, >31µg/dL	No data
		Adults	*Inadequate*	Stillbirth, endocrine effects, birth defects	Unclear	No data

Note: ADHD - attention deficit hyperactivity disorder; IgE – immunoglobulin E; ALS - amyotrophic lateral sclerosis; ECG - electrocardiography

*Increased serum IgE is associated with hypersensitivity; however, as described in **Section 1.4.3**, increased IgE does not equate to disease.

In children, there is *limited* evidence that blood Pb levels<10µg/dL are associated with changes to an immune-related health outcome such as allergy or increased hypersensitivity, although there is *sufficient* evidence that blood Pb levels of 10µg/dL and below are associated with elevated serum immunoglobulin E (IgE) levels. Six studies with mean blood Pb levels <10µg/dL support the relationship between blood Pb and increased serum IgE. Although increases in serum levels of total IgE are not definitive indicators of allergic disease, elevated levels of IgE are primary mediators of hypersensitivity associated with sensitization and allergic disease. Therefore, the studies demonstrating Pb-related increases in IgE suggest a link to hypersensitivity and support more definitive data such as a prospective study that found blood Pb levels <10µg/dL were associated with increased hypersensitivity (or allergy by skin prick testing) in children. These data support the conclusion of *limited* evidence that increased hypersensitivity responses or allergy are associated with blood Pb levels <10µg/dL in children; however there is *inadequate* evidence of an association between blood Pb and other allergic diseases such as eczema or asthma.

There is *inadequate* evidence in adults to address the potential association between blood Pb <10µg/dL and IgE, allergy, eczema, or asthma. Few studies have investigated the relationship between immune function and Pb in humans, and most studies reported general observational markers of immunity rather than function. There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with observational immune endpoints such as altered lymphocyte counts or serum levels of IgG, IgM, or IgA in the peripheral blood of children or adults because of a general lack of studies at the lower dose and inconsistency in available data. There is also *inadequate* evidence that blood Pb levels <10µg/dL are associated with changes in immune function other than hypersensitivity because there are few studies of immune function at lower blood Pb levels.

Bone Pb levels may be particularly relevant for cells of the immune system and immune function. All of the white blood cells or leukocytes that develop postnatally are derived from progenitor cells in the bone marrow. Unfortunately very few studies of immune endpoints have exposure metrics other than blood Pb and therefore, the relative importance of blood or bone Pb levels for immune effects of Pb is unknown.

1.4.4 Cardiovascular Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <10µg/dL in adults are associated with adverse effects on cardiovascular function and that there is *inadequate* evidence to evaluate cardiovascular effects in children (see **Table 1.2** for summary of effects).

There is *sufficient* evidence of a bone Pb-related increase in the risk of hypertension and elevations in blood pressure (BP). Two prospective studies and five cross-sectional studies support a significant association between bone Pb and BP or hypertension in populations with blood Pb levels below 10µg/dL. Studies of blood Pb show less consistent associations with BP and hypertension than bone Pb; however, most of the recent studies with mean blood Pb levels <5µg/dL found significant associations between concurrent blood Pb levels and increased BP. There is *sufficient* evidence of increased risk of mortality from cardiovascular causes associated

with blood Pb levels <10µg/dL, supported by two prospective studies and one prospective study of bone Pb when concurrent blood Pb levels were below 10µg/dL. There is *sufficient* evidence that blood Pb levels <10µg/dL increase the risk of gestational hypertension, supported by one prospective study and five cross-sectional studies with blood Pb levels during pregnancy <10µg/dL. There is *limited* evidence for Pb effects on other cardiovascular outcomes including heart rate variability, electrocardiography (ECG) abnormalities, and clinical cardiovascular disease primarily due to lack of replication studies. Chronic Pb exposure appears to be more critical than current Pb exposure as indicated by more consistent associations between chronic cardiovascular effects and bone Pb as compared to blood Pb. Studies support an association with concurrent blood Pb levels; however, the potential effect of early-life blood Pb levels on cardiovascular outcomes in adults cannot be discriminated from the effect of concurrent blood Pb levels without additional prospective studies in a population for which blood Pb levels remain consistently below 10µg/dL from birth until evaluation of the various cardiovascular outcomes.

There is *inadequate* evidence to assess whether children or menopausal women present a sensitive life stage for cardiovascular effects of Pb. No prospective studies have followed children with early-life Pb measures and evaluated cardiovascular health after childhood. During menopause and with osteoporosis, bone Pb stores are mobilized, thereby increasing circulating Pb levels. Post-menopausal women have not been sufficiently studied to enable conclusions on Pb-related cardiovascular health risks.

1.4.5 Renal Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with adverse renal effects in adults (see **Table 1.2** for summary of effects). There is *limited* evidence that blood Pb levels <5µg/dL are associated with adverse renal effects in children age 12 and older, and the current evidence is *inadequate* to conclude that blood Pb <10µg/dL is associated with renal effects in children below 12 years of age.

There is *sufficient* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in adults. Most of the 13 epidemiological studies of the general population reported positive associations between blood Pb levels below 10µg/dL and increased risk of chronic kidney disease and negative associations with estimated glomerular filtration rate (eGFR) and creatinine clearance. The associations are typically stronger in certain sub-populations, namely hypertensives and diabetics. Comparatively few studies have examined markers of exposure other than blood Pb levels; therefore, it is unknown if blood or bone Pb levels would be a better exposure metric for Pb-related kidney effects. Epidemiological data from the general population support an association with concurrent blood Pb levels; however, the potential effect of early-life blood Pb levels on kidney function in adults cannot be discriminated from the effect of concurrent blood Pb levels without additional prospective studies in a population for which blood Pb levels remain consistently below 10µg/dL from birth until evaluation of kidney function.

There is *inadequate* evidence available to address the potential association between low-level blood Pb in children under the age of 12 and impaired kidney function, but *limited* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in children age 12 and older. There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with kidney function in children under the age of 12 because of inconsistent results and studies lacking clear predictive measures of kidney function in young children. The conclusion of *limited* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in children age 12 and older is based on one study of NHANES data reporting effects that are consistent with reduced eGFR reported in adults in several NHANES studies.

1.4.6 Reproduction and Developmental Effects

The NTP concludes that there is *sufficient* evidence that blood Pb levels <10µg/dL are associated with adverse health effects on development in children and reproduction in adult women (see **Table 1.2** for summary of effects).

Because the database of human studies on most reproductive endpoints is limited to occupational exposure studies, many of the available studies are for blood Pb levels >10µg/dL. Given this fact and the focus of the original nomination on reproductive and developmental effects, higher blood Pb levels were included in the evaluation of these health effects unlike other sections of this document. Consideration of blood Pb levels >10µg/dL resulted in several conclusions for Pb-related reproductive effects in men, but did not affect the conclusions for women or children.

Unlike the dataset for most other health outcomes, there are a number of prospective studies of developmental effects that include prenatal exposure metrics (either maternal blood or cord blood). These prospective studies provide *limited* evidence that prenatal exposure to blood Pb levels <10µg/dL are associated with reduced postnatal growth in children. Conclusions on effects of prenatal exposure for outcomes evaluated as children are complicated by the high degree of correlation in childhood blood Pb levels over time, and as described below, concurrent blood Pb levels <10µg/dL in children are also associated with reduced postnatal growth.

In children, there is *sufficient* evidence that blood Pb levels <10µg/dL are associated with delayed puberty and *limited* evidence for this effect at blood Pb levels <5µg/dL. Nine studies reported that concurrent blood Pb levels <10µg/dL in children are associated with delayed puberty. There is *sufficient* evidence that blood Pb levels <10µg/dL are associated with decreased postnatal growth. Numerous cross-sectional studies, including studies with large sample sizes such as the NHANES datasets, reported that concurrent blood Pb <10µg/dL in children is associated with reduced head circumference, height, or other indicators of growth.

In adults, there is *sufficient* evidence that maternal blood Pb levels <10µg/dL are associated with reduced fetal growth or lower birth weight. Three prospective studies with maternal blood Pb data during pregnancy, a large retrospective cohort of over 43000 mother-infant pairs, and a number of cross-sectional studies with maternal or cord blood Pb at delivery

support an association between higher blood Pb and reduced fetal growth at mean blood Pb levels from 1 to 10µg/dL. Although maternal or paternal bone Pb data are not available in studies of most reproductive health outcomes, a set of studies from a single population reported that maternal bone Pb is negatively related to fetal growth. There is also *limited* evidence that maternal blood Pb levels <10µg/dL are associated with preterm birth and spontaneous abortion. Although several prospective studies reported an association between maternal blood Pb and preterm birth, the conclusion of *limited* evidence is due to inconsistent results and because a retrospective study with a large cohort of over 43000 mother-infant pairs did not find an association between maternal blood Pb levels and preterm birth. The conclusion of *limited* evidence for an association with spontaneous abortion is based primarily on the strength of a single nested prospective case-control study in women with additional support provided by occupational studies that reported an association with Pb exposure but lack blood Pb measurements. In men, there is *inadequate* evidence that blood Pb levels <10µg/dL are associated with effects on reproduction.

In men there is *sufficient* evidence that blood Pb levels ≥15µg/dL are associated with adverse effects on sperm or semen and blood Pb levels ≥20µg/dL are associated with delayed conception time. Decreased sperm count, density, and concentration have been reported in multiple retrospective and cross-sectional occupational studies of men with mean blood Pb levels from 15-68µg/dL. Four studies reported increased time to pregnancy in women whose male partners had blood Pb levels of 20-40µg/dL. A single retrospective occupational study reported increased risk of infertility among men with blood Pb levels ≥10µg/dL, and the continuity of these data with effects on time to pregnancy supports a conclusion of *limited* evidence that blood Pb levels ≥10µg/dL in men are associated with other measures of reduced fertility. There is also *limited* evidence that paternal blood Pb levels >31µg/dL are associated with spontaneous abortion. Similar to the evidence for maternal effects, the conclusion of *limited* evidence in men is based primarily on the strength of a single nested retrospective case-control study in men with additional support provided by occupational studies that reported an association with Pb exposure but lack blood Pb measurements.

1.5 Future Research

There are strong data and *sufficient* evidence for adverse health effects across a wide range of health outcomes in children and adults at blood Pb levels <10µg/dL as described above. Over time, epidemiological studies have provided data to support health effects at lower and lower blood Pb levels, particularly in children. Prospective studies in children can better address the lower limits of Pb exposure associated with health effects because they can assure that the blood Pb levels remain below a given value (i.e., <10µg/dL or <5µg/dL) throughout life. Studies of health effects in adults cannot eliminate the potential effects of early-life blood Pb levels on health effects observed as adults. This is particularly important in an evaluation of the health effects of blood Pb levels <10µg/dL because older adults were likely to have had a blood Pb level above 10µg/dL as children (see discussion in **Section 1.3**), whereas only 0.8% of children had confirmed blood Pb levels over 10µg/dL in 2008.

The clarification of the effects of early-life blood Pb levels relative to the effects of concurrent blood Pb levels remains a significant issue for evaluating Pb-related health effects in adults. Epidemiological data from adults support an association between concurrent blood Pb levels <5µg/dL and decreased renal function, and that concurrent blood Pb levels <10µg/dL are associated with increased blood pressure, hypertension, and increased cardiovascular-related mortality. Long term prospective studies in a population for which the data demonstrate that blood Pb levels remained consistently below 10µg/dL from birth until the outcome of interest is measured would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL.

2.0 METHODS

The NTP's conclusions on health effects of low-level Pb are based on evaluation of data from epidemiological studies with a focus on blood Pb levels <10μg/dL. The methodological approach began with a statement of the key questions addressed by this evaluation. The general approach for developing the NTP's conclusions on evidence of an association between blood Pb levels <10μg/dL and specific health effects are described below along with the format and definitions used throughout the document. The structure of appendix tables summarizing the relevant literature for each health effect category is also described below. The NTP considered several recent government evaluations of the health effects of Pb to supplement a review of the primary epidemiological literature and these documents are briefly described in this section. The literature search strategy and the peer review process are also described below.

2.1 Key Questions

What is the evidence that adverse health effects are associated with blood lead <10μg/dL?

- What reproductive, developmental, neurological, immune, cardiovascular, and renal health effect(s) are associated with blood lead levels <10μg/dL?
- What is the blood lead level associated with the health effect (i.e., <10μg/dL or <5μg/dL)?
- At which life stages (childhood, or adulthood) is the effect identified?
- Are there data to evaluate the association between bone Pb and the health effect and how does the association to this biomarker of Pb exposure compare to the association with blood Pb?

2.2 Approach to Develop Health Effects Conclusions

Conclusions in the NTP evaluation of Pb-related health effects in humans associated with low-level Pb were derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10μg/dL. The evaluation includes a review of the primary epidemiological literature for evidence that low-level Pb is associated with the following: neurological effects, immunological effects, cardiovascular effects, renal effects, and reproductive and developmental effects. These health effects were selected because there is a relatively large database of human studies in each area. The NTP considered four possible conclusions for specific health effects in each area:

> **Sufficient Evidence of an Association**: a relationship is observed between the exposure and health outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.
>
> **Limited Evidence of an Association**: an association is observed between the exposure and health outcome for which a causal interpretation is credible, but chance, bias, and confounding could not be ruled out with reasonable confidence.

Inadequate Evidence of an Association: the available studies are insufficient in quality, consistency, or statistical power to permit a conclusion regarding the presence of absence of an association between exposure and health outcome, or no data in humans are available.

Evidence of No Association: There are several adequate studies covering the full range of levels of exposure that humans are known to encounter (in this case limited to blood Pb levels <10µg/dL), which are mutually consistent in not showing an association between exposure to the agent and any studied endpoint.

The discussion of each health effect begins with a statement of the NTP's conclusion regarding whether the specific effect is associated with a blood Pb level <10µg/dL or <5µg/dL and the age group in which it is, or is not identified (childhood or adulthood) as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. For the purposes of this evaluation children refers to individuals <18 years of age unless otherwise specified. A lower effect level of 5µg/dL was selected because it is commonly used in epidemiological studies to dichotomize health-effects data by exposure levels and, therefore, data are often available to evaluate health effects for groups above and below this value. Key data and principal studies considered in developing the NTP's conclusions are then discussed in detail. Findings described in the text as having an association or significant association both reflect a statistically significant result with a *p*-value <0.05 unless otherwise indicated. Each section concludes with a summary discussing each health effect providing experimental animal data that pertain to the human evidence, and stating the basis for the NTP's conclusions.

2.3 Appendices of Studies Considered:

The information to support the NTP's conclusions for individual health effects is presented in each chapter. Human studies from populations with low-level Pb exposure that were considered in developing the conclusions are also abstracted for further reference and included in separate appendices for each category of health effect.

Each appendix table includes the following column headings:

Description: study design, reference, and geographic location
Population: sample size, description, years of study, and percent male
Age: mean age and standard deviation of the subjects
Blood Pb: mean blood Pb level and standard deviation in µg/dL (and bone Pb levels)
Outcomes: health effects assessed
Statistical: methods used and cofactors included in analyses
Findings: results summary (bolded if statistical significance tests had a *p*-value <0.05)
Observed effect: conclusion (Supporting/Not Supporting/Equivocal) and description

Potential overlap of subjects in multiple publications from the same epidemiological study is indicated in the first column. These studies were not considered as independent findings supporting (or not supporting) the NTP's conclusions.

The grouping of studies within the appendix tables varied by health effects considered:

Appendix A. Neurological Effects: no grouping, meta-analyses shaded
Appendix B. Immune Effects: grouped by low (<15) and high (>15 exposure)
Appendix C. Cardiovascular Effects: grouped by outcome, meta-analyses shaded
Appendix D. Renal Effects: no grouping
Appendix E. Reproductive and Developmental Effects: grouped by outcome

Within each grouping, studies are listed alphabetically by first author and then chronologically by publication date. For the appendix tables grouped by outcome, if a publication contained results in more than one group, results were included specific to each outcome group.

The NTP's conclusions are based on the evidence from human studies with blood Pb levels of <10µg/dL and therefore the abstracted studies in the appendices are principally those with a mean blood Pb level of <10µg/dL. However, studies with data reflecting mean exposure levels up to 15µg/dL were also included so that effects at and around 10µg/dL were not missed during the evaluation. Reproductive effects in studies with mean blood Pb levels over 15µg/dL were included in the evaluation because there is a limited dataset of human studies associated with lower blood Pb levels. The immunological effects database was adequate to make conclusions on several effects at blood Pb levels <10µg/dL and the NTP makes limited reference to studies in humans at higher blood Pb levels. Therefore, Appendix B includes human studies with higher blood Pb levels (i.e., >15µg/dL).

2.4 Authoritative Sources Considered

Recent government evaluations of the health effects of Pb include the US EPA 2006 Air Quality Criteria Document (AQCD) for Lead (U.S. EPA 2006), the ATSDR 2007 Toxicological Profile for Lead (ATSDR 2007), and the CDC's Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) Reports such as the 2010 Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women (CDC 2010). The NTP made extensive use of these evaluations in the current assessment, especially the EPA's 2006 AQCD for Lead because it underwent extensive external public peer-review. NTP considered the conclusions and data summaries from the EPA and ATSDR documents. In general, NTP concurred with the conclusions and agreed that the data support them. Differences between the NTP's conclusions and the 2007 ATSDR Toxicological Profile for Lead and the EPA's 2006 AQCD are identified for specific endpoints in the document. The database of studies on health effects in humans is supported by an equally large body of experimental animal studies. In this document, the experimental animal data are considered when relevant to reaching conclusions primarily based on the human literature. The reader is referred to the US EPA AQCD for Lead (U.S. EPA 2006) and ATSDR Toxicological Profile for Lead (ATSDR 2007) for more in-depth reviews of the animal data.

2.4.1 US EPA 2006 Air Quality Criteria Document (AQCD) for Lead

The EPA's AQCD is an exhaustive review (over 1,200 pages with an additional 900 pages of tables and other annex material) and assessment of the scientific information related to human

health and ecological effects associated with lead in ambient air (U.S. EPA 2006). The EPA's AQCDs are published periodically (the latest draft document, released May of 2011 updates the review with literature published since the US EPA 2006 AQCD for Lead to provide key scientific assessment of evidence to support periodic review of the current Pb National Ambient Air Quality Standards (NAAQS). The 2006 EPA AQCD for Lead is an extensively reviewed document that was subject to public comment and review by the Clean Air Scientific Advisory Committee (CASAC) in a series of public meetings. EPA is in the process of revising the AQCD and the 2011 Integrated Science Assessment (ISA) for Lead (U.S. EPA 2011) is only available as a draft at this time.

2.4.2 ATSDR 2007 Toxicological Profile for Lead

The 2007 Toxicological Profile for Lead (ATSDR 2007) is a comprehensive evaluation of the available toxicological and epidemiological data on lead. The toxicological profile is organized around a public health statement summarizing the toxicological and adverse health effects for lead. ATSDR's peer review process for their Toxicological Profiles includes release for public comment and a peer review by a panel of experts.

2.4.3 CDC Lead Panel Documents

CDC's 5th revision of the statement on preventing lead poisoning in young children includes a companion document developed by the Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) which reviews the scientific evidence for adverse health effects in children at blood lead levels below 10 μg/dL. The committee concluded the "overall weight of the evidence supports an inverse (negative) association between BLLs [blood lead levels] <10μg/dL and the cognitive function in children" (CDC 2005). The report primarily focuses on cognitive function but the committee also concluded that there were additional health effects (e.g., other neurological functions, stature, sexual maturation) associated with blood Pb levels <10μg/dL in children.

The ACCLPP has also prepared a draft report providing Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women (CDC 2010). The report provides "practical considerations regarding preventing lead exposure during pregnancy, assessment and blood lead testing during pregnancy, medical and environmental management to reduce fetal exposure, breastfeeding and follow up of infants and children exposed to lead *in utero*." The document summarizes the evidence from human studies through 2008 for health effects of lead in pregnant women and on the developing child (concentrating on *in utero* and lactational exposure) and provides guidance for clinicians.

2.5 Literature search strategy

The 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead were screened for citations on health effects assessed at low-level Pb exposure. The NTP's conclusions are based on the evidence from human studies with blood Pb levels of <10μg/dL with data reflecting mean exposure levels up to 15μg/dL also considered so that effects at and around 10μg/dL were not missed during the evaluation. Primary literature searches in MEDLINE®, Web

of Science, Scopus, Embase, and Toxnet were conducted on March 1-5, 2010 to identify relevant studies published subsequent to the 2006 EPA and 2007 ATSDR documents. Search terms included the following MeSH subject headings: lead or lead poisoning; "diseases category" or "anatomy category" for health effects; and humans[mh] or epidemiology[sh] or epidemiologic studies[mh] or age groups[mh] for limiting to human studies.

Due to the heteronym nature of the term 'lead,' textword searching used four approaches. 1) search for lead in title, 2) used various combinations to focus on low-level exposure: "low lead" or "low blood lead" or "lower lead" or "lower blood lead" or "low level" or "low levels" or "lower level" or "lower levels" or "lead level" or "lead levels" or "low dose" or "lead induced" or "lead intake" or "blood lead," 3) combined lead with heavy metals or cadmium or mercury or arsenic and 4) when necessary, excluded "lead to" or "leads to" from search results. For databases that allowed proximity searching, "lead" and "low or lower" were required to be in the same sentence. This strategy would retrieve articles such as "low cadmium and lead levels" or "low blood and urine lead levels" or "lower concentrations of lead in the blood." Textwords used to retrieve human studies included: human(s), resident(s), inhabitant(s), population, people, subject(s), patient(s), case(s), women, men, girls, boys, parent(s), mother(s), father(s), adult(s), child, children, childhood, adolescent(s), infant(s), toddler(s), newborn(s), occupation(al), work, workplace, worker(s), employee(s), laborer(s), and staff.

An updated search was performed from September 12-15, 2011 to identify any additional references published since the last search. Technical advisors who were involved in the review of the draft document (see below) were also asked to identify relevant studies. In addition, NTP published a Federal Register notice regarding the low-level lead evaluation inviting submission of information about recently published/in-press studies that might be relevant for consideration in the evaluation (75 FR 51815).

2.6 Peer Review Process

The primary mechanism for obtaining scientific input during development of the draft NTP Monograph on Health Effects of Low-Level Lead was through technical advisors (see **Contributors** for list of technical advisors). These advisors were asked to provide input on issues of scientific complexity, adequacy of the literature review, and overall presentation of a pre-public release version of the draft NTP monograph. Individuals who served as technical advisors were screened for potential conflict of interest.

Peer review of the draft NTP Monograph on Health Effects of Low-Level Lead will be conducted by an expert panel of *ad hoc* reviewers with relevant scientific background (i.e., expertise in Pb or metals related reproductive and developmental toxicology, neurotoxicology, immunotoxicology, cardiovascular toxicology, renal toxicology, and exposure) at a public meeting scheduled for November 17-18, 2011. The selection of panel members and conduct of the peer review will be in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel will be charged to determine whether the science cited in the draft NTP Monograph on Low-level Lead is technically correct, clearly stated, and supports the NTP's conclusions regarding the potential for adverse health effects to occur at blood lead

levels <10μg/dL. Written public comments received on this draft and comments from peer reviewers will be considered during finalization of the document (see *http://ntp.niehs.nih.gov/go/36639* for updates).

3.0 EXPOSURE

Studies of health effects of Pb in humans commonly utilize one of several biomarkers to reflect the level of Pb exposure in an individual. The overwhelming majority of studies measure whole blood Pb as blood samples are routinely collected and stored in large epidemiological studies; furthermore, the methods for measuring Pb in whole blood are widespread and extensively validated. Bone Pb is more likely to reflect cumulative exposure, but must be measured by specialized equipment and requires measurements to be made on subjects present at a research clinic. Measures of Pb in urine and hair have been used in some studies, but their reflection of the body burden of Pb is less clear.

Pb is ubiquitous in the environment, but the level of exposure to Pb that individuals experience can vary and depend on many factors, including occupation, geography and life stage. This chapter briefly discusses common routes of exposure to Pb and associated factors that may affect the risk of exposure.

The NTP made extensive use of recent government evaluations of Pb exposure and associated health effects in developing the current assessment. The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both contain extensive review and discussion of Pb exposure with particular focus on air Pb in the AQCD document. The NTP also used two CDC documents focused on particularly vulnerable populations: the 2010 Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women and the 2005 Preventing Lead Poisoning in Young Children report (CDC 2005b, 2010). The EPA is in the process of revising the AQCD, and has released an external review draft that also includes extensive discussion of Pb exposures (U.S. EPA 2011).

3.1 What Does It Mean to Refer to Blood Pb <10µg/dL?

This evaluation focuses on the relationship between health effects and blood Pb levels <10µg/dL because whole blood Pb is the most widely available exposure metric, blood Pb reflects an equilibrium between current environmental Pb exposures and Pb stored in bone from prior exposures, and numerous studies have reported an association between blood Pb levels and health outcomes.

However, measuring Pb in one tissue at one point in time does not present a complete picture of either current or cumulative Pb exposure. Bone Pb is considered superior to blood Pb in reflecting the long term stores of Pb in the body (Coon *et al.* 2006); and when available, bone Pb data were also considered in this evaluation. However, measuring bone Pb is expensive and requires subjects to travel the location of the specialized K-x-ray fluorescence apparatus. Blood Pb may provide a better measure of Pb exposure in children or other subjects with active bone remodeling (Barbosa *et al.* 2005; Hu *et al.* 2007).

Pb exposures in the United States have dramatically declined over the last 30 years after bans on Pb in paint, solder, and gasoline; representing a significant public health accomplishment and protection for current populations of children. However, children born in the United States

in the 1970's had a mean blood Pb of 15µg/dL during early childhood. Consequently, health effects in adults today may have been influenced by blood Pb levels >10µg/dL that many individuals experienced earlier in life.

Remaining cognizant of these childhood blood Pb levels, there are data on multiple health effects in adults for which studies report a significant relationship with concurrent blood Pb levels (e.g., elevated blood pressure, reduced kidney function, or decreases in specific measures of cognitive function). There is a considerable body of evidence that these health effects are associated with Pb exposure and multiple studies report a significant association with concurrent blood Pb levels <10µg/dL. Furthermore the association with blood Pb is supported by the consistency of effects across epidemiological studies and biological coherence with animal data. It is well recognized that the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb without additional long term studies.

As described in **Section 2.2**, the NTP's conclusions were derived by evaluating data from epidemiological studies with a focus on blood Pb levels <10µg/dL. The evidence is discussed for specific health outcomes within each chapter, and varies by the study design and type of analysis used to examine the relationship of the health outcome with blood Pb across the hundreds of studies evaluated. In some cases, authors restricted the analysis to the population with blood Pb levels <10µg/dL, <5µg/dL, or even lower and the association of the health effect with the blood Pb level is clear. For example, Lanphear *et al.* (2000) reported an inverse relationship between blood Pb and academic performance in a cross-sectional study of 4853 children ages 6-16 from the NHANES III dataset; the association with blood Pb remained significant in further analyses restricted to 4681 children with blood Pb <10µg/dL ($p<0.001$), and 4043 children with blood Pb <5µg/dL. In other cases, the authors reported a significant association between blood Pb and an effect in a population with a mean blood Pb level <10µg/dL (e.g., blood Pb level is associated with higher blood pressure in a study of 964 adults in the Baltimore Memory Study (Martin *et al.* 2006)). These analyses support an effect of a blood Pb level <10µg/dL, but they do not exclude the possibility that individuals significantly above or below the mean blood Pb level could be driving the effect. Finally, in some studies, the authors compared effects between a Pb-exposed population and internal or external referents with lower blood Pb levels. For example, Naicker *et al.* (2010) compared the effect of a blood Pb level ≥5µg/dL with a blood Pb level <5µg/dL on developmental markers of puberty in a study of 682 girls age 13 in South Africa and found blood Pb ≥5µg/dL to be significantly associated with breast development, pubic hair development, and age of menarche.

3.2 Biomarkers of Pb Exposure

The large majority of human epidemiological studies reporting individual exposure levels measured Pb in blood samples. This chapter discusses US blood Pb levels and trends for age, gender, and race or ethnicity. Bone Pb has been measured in some studies and is considered a more accurate reflection of cumulative body burden of Pb due to the longer half-life of Pb in bone relative to blood (Coon *et al.* 2006). Bone and blood Pb are currently the most useful

tools for measuring the body burden of Pb, while measures of Pb in urine and hair are less commonly used and are of low utility (Hu *et al.* 2007).

While whole blood Pb is the most readily available biomarker for Pb exposure (and is the basis for this evaluation of Pb levels below 10µg/dL), plasma Pb is the portion available to cross cell membranes into specific tissues of the body (Cavalleri *et al.* 1978). Plasma Pb represents less than 5% of the whole blood Pb concentration, but the proportion of whole blood Pb in plasma Pb can vary widely and be influenced by bone Pb levels (Hernandez-Avila *et al.* 1998; Hu *et al.* 1998). Measuring plasma Pb is technically difficult, requires specialized equipment not widely available, and is not typically measured in research or clinical settings (CDC 2010).

The National Health and Nutrition Examination Surveys (NHANES) include whole blood Pb measurements on a cross-section of the US population. Specific outcomes in subsets of the study are routinely published and included in the chapters of this document covering specific health effects. General trends in blood Pb levels from NHANES data are presented in Figures 7.1, 7.2, and 7.3 (from Mahaffey *et al.* (1982), Brody, *et al.* (1994), and the CDC (2005a, 2011b) including the Updated Tables for (CDC 2009b)).

Blood Pb levels have decreased between over the last 30 years for all age groups (see **Figure 3.1**). The declining blood Pb levels follow declines in Pb exposure related to bans on leaded gasoline, paint, and use of solder in food cans and plumbing in the United States (see **Section 3.3 Sources of Pb**).

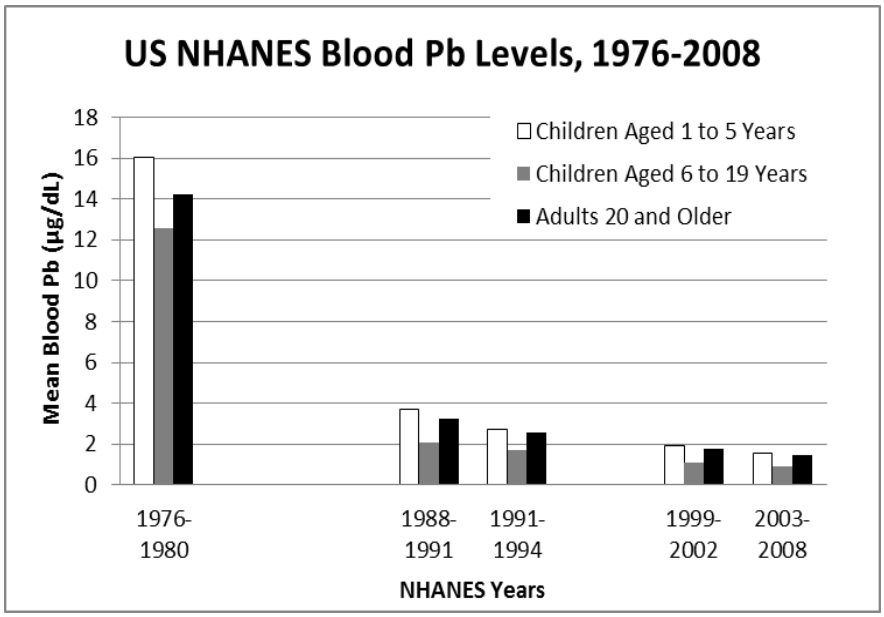

Figure 3.1 US NHANES blood Pb levels for children and adults from 1976-1980 (Mahaffey *et al.* 1982), 1988-1991 (Brody *et al.* 1994), 1991-1994, 1999-2002 (CDC 2005a), and 2003-2008 (CDC 2011b). Years and ages grouped based on available published data.

Unfortunately, the burden of Pb exposure is not uniformly low in all racial and ethnic subpopulations (see **Figure 3.2**). Non-Hispanic blacks have higher blood Pb levels than non-Hispanic whites across all ages, and being non-Hispanic black is a major risk

factor for higher Pb levels in children (Jones *et al.* 2009). When comparing levels of Non-Hispanic blacks to those of Non-Hispanic whites almost every age and gender group was statistically significantly higher among blacks in both 1999-2002 and earlier in 1991-1994 (CDC 2005a).

Males also consistently have higher blood Pb levels than females (see **Figure 3.3**), and this trend was observed in NHANES across most age groups and all racial/ethnic groups (CDC 2005a).

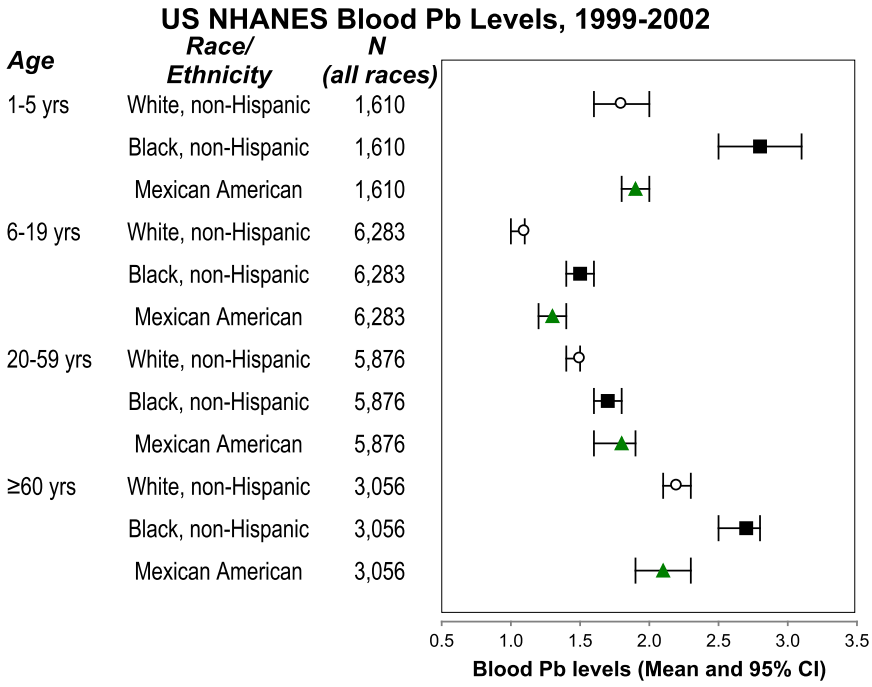

Figure 3.2 NHANES 1999-2002 mean blood Pb level and 95% CI for each age category for non-Hispanic white (open circles), non-Hispanic black (filled squares), and Mexican Americans (green triangles) (CDC 2005a)

Given an accumulating body burden of Pb and higher past levels of Pb exposure, blood Pb levels are expected to go up with age; however, young children (ages 1-5 years) consistently have higher blood Pb levels than older children, likely due to hand-to-mouth behavior in this age group (see **Figure 3.2**). Several studies show a peak in children's blood Pb levels around 24 months of age (CDC 2007). Children are the focus of several blood Pb screening and exposure reduction programs because of these higher levels and established developmental impairments from Pb exposure (e.g., CDC's Childhood Lead Poisoning Prevention Program see **http://www.cdc.gov/nceh/lead/about/program.htm**) (CDC 2005b; Clark *et al.* 2011). Children's blood Pb levels (ages 1-5 years) have decreased 10 fold over the last 30 years (Geometric mean

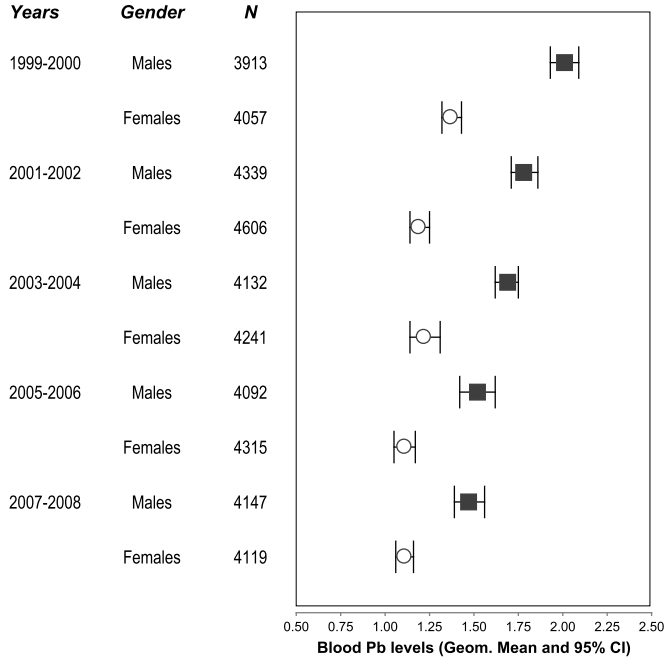

Figure 3.3 NHANES 1999-2008 mean blood Pb level and 95% CI for all ages for men (squares) and women (circles) (CDC 2011b)

1976-1980: 15.1µg/dL; 2007-2008: 1.51µg/dL (CDC 2007, 2011b)).

In 2008, only 0.8% of children had confirmed blood Pb levels over 10µg/dL, down from 7.6% in 1997 (**http://www.cdc.gov/nceh/lead/data/national.htm**). However, blood Pb levels have remained consistently higher in non-Hispanic black children, which may be linked to a variety of factors contributing to higher Pb exposure such as lower socioeconomic status, living in older, urban housing, or having lower calcium intake (see **Figure 3.2**, discussed further in **Section 3.3 Sources of Pb** and **Section 3.4 Modifiers of Pb Exposure**) (Haley and Talbot 2004). Pb exposure in this critical developmental period can have immediate impacts on children's health and contribute to a lifetime of exposure from Pb stored in bone.

An individual's blood Pb level reflects an equilibrium between current exogenous environmental Pb exposure and the endogenous body burden of Pb (Factor-Litvak *et al.* 1999). The body quickly eliminates metals from circulating blood, while bone is a repository for Pb and more accurately reflects the cumulative dose of Pb integrated over years or even decades (Coon *et al.* 2006; Hu *et al.* 1998). The half-life of Pb in blood is approximately one month, while the half-life in bone ranges from 10-30 years depending on the bone turnover rate, which varies by type of bone and life stage (Rabinowitz 1991). It is estimated that 45-70% of blood Pb comes from Pb released from endogenous tissue Pb stores, primarily in bone (Gulson *et al.* 1995).

The distribution of Pb in tissues changes with life stage. In adults, bone and teeth store 90-95% of the total body burden of Pb, while it is ranges from 70-95% in children (Barbosa *et al.* 2005; Hu *et al.* 2007). Bone Pb was found to be the source of between 40 and 70% of blood Pb in individuals undergoing hip or knee replacement surgery (Smith *et al.* 1996). Pregnancy, lactation, menopause, and osteoporosis mobilize bone stores of Pb and contribute to increased Pb exposure to other tissues in the body or a developing fetus from Pb released from maternal bone (Hu *et al.* 2007; Manton *et al.* 2003; Silbergeld *et al.* 1988). In young children, continuous growth results in constant bone remodeling and bone Pb is exchanged with blood Pb much more frequently than in adults (Barbosa *et al.* 2005; Hu *et al.* 2007). Additional factors that can increase risks to women and children are discussed further in the **Section 3.4 Modifiers of Pb Exposure**.

Bone Pb is typically measured by K-x-ray fluorescence (KXRF); however, there are few research institutions that possess this technology and trained staff. Recently, a portable XRF device for *in vivo* bone Pb measures was developed that may be feasible for use in future studies (Nie *et al.* 2011). Another modeling approach estimates bone Pb levels from blood Pb measures and covariates typically collected in epidemiological studies (Park *et al.* 2009). This approach could be used to estimate bone Pb in existing studies without the ability to measure bone Pb directly. While blood Pb is by far the most common measure of exposure, it may not be as appropriate as bone Pb, particularly for studies of chronic health conditions (Coon *et al.* 2006; Hu *et al.* 2007). Physiologically based pharmacokinetic (PBPK) models have been created to combine current blood and bone Pb measures to estimate the Pb levels at the time of the exposure,

allowing a more complete model of the individual's lifetime Pb exposure (Coon *et al.* 2006; Leggett 1993).

Pb has also been measured in other materials that are easier to obtain; but can fluctuate temporally, as blood Pb does. Hair collection is minimally invasive and easier to ship and store, but does not have standardized protocols and hair is subject to contamination from environmental sources of Pb (Barbosa *et al.* 2005; Harkins and Susten 2003; Seidel *et al.* 2001). In 2001 an ATSDR expert panel concluded that there were too many unresolved scientific issues for hair to be a useful source for evaluating trace metal exposures including Pb (ATSDR 2001). Collection of urine is non-invasive, and urine has also been used to measure Pb; however, urine Pb levels vary rapidly and independently of blood Pb and require creatinine and glomerular filtration corrections to estimate plasma Pb levels at a specific collection time (Barbosa *et al.* 2005; Hu *et al.* 2007). Fecal Pb levels reflect both excreted biliary Pb and unabsorbed ingested Pb, but must be completely collected over several days to accurately reflect Pb exposures (Barbosa *et al.* 2005).

Unlike these fluctuating measures, teeth accumulate Pb like other bone, lose Pb at a slower rate than other bone, and for childhood exposure studies primary teeth are readily available when lost after age 6 (Manea-Krichten *et al.* 1991). In addition, the layers of the tooth provide a timeline of Pb exposure including *in utero* (enamel) and early childhood (primary tooth dentin) exposures, which may be separately measurable by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) without removing the tooth (Barbosa *et al.* 2005; Uryu *et al.* 2003).

Some studies used indirect measures to estimate Pb exposure, although this is less common as whole blood Pb measurement has become more widespread. Pb inhibits cytoplasmic enzyme δ-aminolevulinic acid dehydratase (ALAD) which is responsible for heme biosynthesis. ALAD can be measured in urine, blood, and plasma and is inversely related to Pb levels (Barbosa *et al.* 2005). While not widely used, ALAD levels in blood may be a better marker of long term exposure than blood Pb measures, but urine ALAD is not sensitive and therefore not a good indicator at low Pb exposure levels (Alessio *et al.* 1981; Telisman *et al.* 1982). Pb can also impair heme formation by inhibiting ferrochelatase such that zinc is used in place of iron, increasing levels of zinc protoporphyrin (ZPP) (Barbosa *et al.* 2005). ZPP levels in blood have been used as an indicator of Pb poisoning, but ZPP testing is not sensitive when blood Pb levels are below 25µg/dL (Labbe *et al.* 1999; Parsons *et al.* 1991; Wildt *et al.* 1987).

Pb is cycled through body tissues via several metabolic processes that are influenced by development and life events. Measuring Pb in one tissue at one point in time does not present a complete picture of cumulative Pb exposure, but bone Pb is superior to blood Pb in reflecting the long term stores of Pb in the body (Coon *et al.* 2006). However, measuring bone Pb is expensive, requires specialized equipment not widely available, requires study subjects to travel to the location of the K-x-ray fluorescence apparatus, and cannot be done retrospectively on stored samples from large epidemiological studies.

3.3 Sources of Pb

In the general population, the primary route is oral exposure to Pb from ingesting contaminated food and water or inhaling air and soil containing Pb. For an extensive discussion of environmental sources of Pb, see the EPA's 2006 Air Quality Criteria Document (U.S. EPA 2006). Hand-to-mouth behavior in young children increases their risk of exposure to Pb in dust, toys and paint. Occupational exposures in Pb industries are often associated with elevated Pb levels in workers, and can also contribute to Pb exposures in coworkers who do not work with Pb or family members exposed to dust brought into the home from the person who works with Pb (Hipkins *et al.* 2004).

Dietary Pb sources in the United States have been reduced through several changes in practice, such as removing Pb solder from cans and banning Pb-arsenate pesticides (Bolger *et al.* 1996), and current Pb levels in the US food supply are low (CDC 2010). Contaminated food, particularly if imported from other countries, can be a source of dietary Pb exposure. A study of pregnant women in Monterey, California identified prepared grasshoppers sent from Oaxaca, Mexico as a source of Pb poisoning (Handley *et al.* 2007) and tamarind candies imported from Mexico were linked to several cases of Pb poisoning in children (CDC 2002). Spices, herbs, nutritional supplements, and traditional medicines have been shown to contain or be contaminated with Pb as well (Buettner *et al.* 2009; CDC 1999, 2002; Ko 1998; Lin *et al.* 2010). Pottery with a Pb glaze can contaminate food if used for cooking or storage (CDC 2010). While high, acute exposures have been reported from Pb from pottery leaching into food (Matte *et al.* 1994); long term use may cause a low, chronic exposure and raise the body burden of Pb (Hernandez Avila *et al.* 1991). Use of Pb-glazed ceramics was a major source of cumulative Pb exposure in a study of women in Mexico (Brown *et al.* 2000). Pb crystal can elute Pb into alcoholic beverages at levels above the EPA's maximum allowable level for drinking water (Graziano and Blum 1991). Approximately 25% of home-distilled alcohol (moonshine) samples tested by the US Bureau of Alcohol Tobacco and Firearms between 1995 and 2001 had Pb concentrations >400µg/dL, high enough to produce blood Pb levels over 25µg/dL if one liter was consumed (Morgan *et al.* 2004). Moonshine consumption has been significantly associated with blood Pb levels over 15µg/dL and Pb-related deaths (Kaufmann *et al.* 2003; Pegues *et al.* 1993).

Tap water once contributed to as much as 10-20% of total Pb exposure in the United States prior to amendments to the Clean Water Act (U.S. EPA 2006), and some older pipes, taps, and pre-1986 pipe solder still contain Pb. Source drinking water rarely contains Pb and the Pb enters tap water through corrosion of Pb from pipes and plumbing fixtures. Corrosion creates exposure from Pb deposits even after previous sources of Pb have been removed from water lines, as well as actual Pb pipes or Pb solder. This corrosion can significantly increase the Pb content in drinking water after changes in water disinfection processes, particularly with use of chloramine (Jean Brown *et al.* 2011; Miranda *et al.* 2007). In a highly publicized incident, the District of Columbia's water supply exceeded the 15µg/L action level for Pb several times between 2000 and 2004 due to corrosion of Pb scales in service pipes after a switch to chloramine to reduce disinfection byproducts (U.S. EPA 2007). Monitoring in DC homes with Pb

service lines found a small increase in the incidence of blood Pb levels over 5µg/dL, but not over the 10µg/dL CDC level of concern for children; however, further analysis showed children with Pb service lines were at risk for blood Pb levels above 10µg/dL even during periods when Pb levels in water were below the action level (CDC 2004; Jean Brown *et al.* 2011). In addition, the incidence of blood Pb levels over 10µg/dL was increased in infants less than 1.3 years old during the DC drinking water event (Edwards *et al.* 2009). Infants may be at an increased risk from contaminated water if they drink infant formula made with tap water, as they typically consume 6 oz/kg of formula daily; and infants may have a higher relative exposure than others in the same household (Bearer 1995).

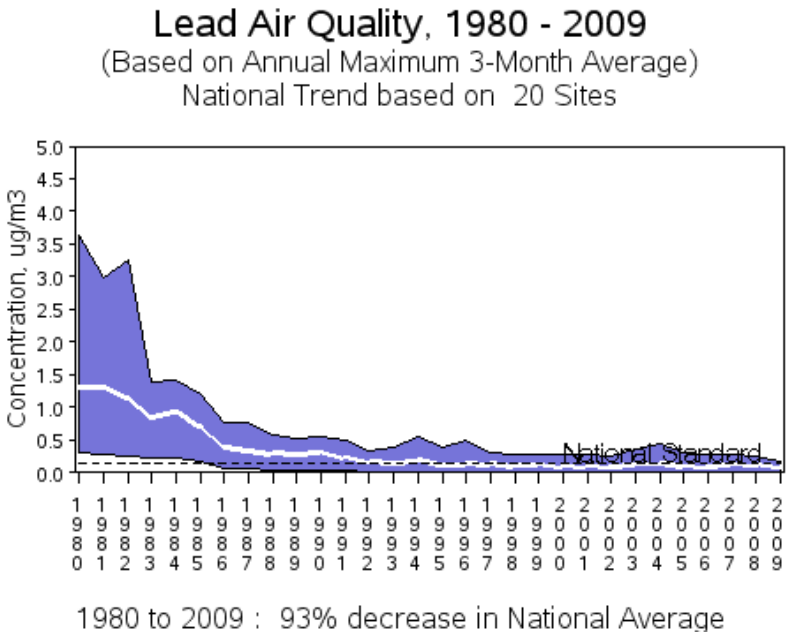

Figure 3.4 US Pb Air Concentration (µg/m^3) from 1980-2009: U.S. Environmental Protection Agency. (http://www.epa.gov/air/airtrends/lead.html, accessed 1 August, 2011)

Inhaled Pb is another source of Pb exposure (U.S. EPA 2006). During the renovation of buildings built before 1978, dust from Pb paint can be inhaled, and residual contamination following a renovation with inadequate cleanup may continue to expose building occupants to Pb. The US Department of Housing and Urban Development estimated that 40% of US housing contains Pb paint, which presents a potential Pb hazard when it is disturbed or deteriorates (Wakefield 2002). Leaded gasoline is another inhaled source of Pb in parts of Asia, Eastern Europe, the Middle East, and South America. In the United States leaded gasoline was banned in 1996 after being phased out for more than 20 years, and average ambient air Pb levels fell 93% between 1980 and 2009 (**Figure 3.4**) (U.S. EPA 2006). While Pb paint and leaded gasoline are no longer major sources of Pb in the United States, Pb from these sources remains in soil and dust as well as in an endogenous body burden from earlier exposures to Pb paint and gasoline (Hu *et al.* 1998).

Smoking or exposure to passive smoke may lead to increased exposure to Pb in environmental tobacco smoke (ETS). Tobacco itself contains Pb, in part at least, from ambient air sources: the levels of Pb in mainstream smoke from Canadian-grown tobacco cigarettes decreased by 62% from 1968 to 1988 as ambient air Pb levels declined (Rickert and Kaiserman 1994). Serum cotinine and postnatal exposure to ETS were significantly associated with blood Pb levels of children in NHANES III; and the levels did not decrease with age, indicating inhalation was more

likely than hand-to-mouth behavior in younger children (Lanphear *et al.* 2000; Mannino *et al.* 2003). In studies of outcomes causally linked to ETS exposure, such as neurodevelopment or cardiovascular disease, ETS may confound the observed associations of Pb and the health outcome (CDC 2005a).

Contaminated soil also contributes to Pb exposure in humans if inhaled as dust or eaten. Ingested Pb from soil is 26% bioavailable when fasting and 2.5% bioavailable after a meal (Maddaloni *et al.* 1998). Clay tablets sold in Mexico, Central America, and parts of Africa are eaten for religious reasons, health promotion, or simply taste and texture (CDC 2010). Children and people with pica (particularly pregnant women) who eat clay and other non-food items can also ingest Pb (Klitzman *et al.* 2002).

Children are most commonly exposed to Pb in paint, household dust, and soil - particularly if they reside in pre-1978, deteriorated housing - and can increase their risk of exposure by natural mouthing tendencies (Lanphear *et al.* 1998). There are few direct data on Pb absorption from toys or other consumer products, but it is clear that Pb can and is absorbed from toys in some cases. Pb concentration in toys is principally associated with use of Pb in paints, coloring agents, and plastic stabilizers in polyvinyl chloride plastics (Godoi *et al.* 2009; Greenway and Gerstenberger 2010). A 4-year-old boy had an extremely high Pb level (123µg/dL blood Pb) after swallowing a vending machine necklace pendant that contained 39% Pb (VanArsdale *et al.* 2004), and similar products could cause lower Pb exposure levels if chewed but not swallowed. Publications have not been identified that quantitate absorption differences relative to Pb in paint or embedded in plastics as a coloring agent or stabilizer. However, Sanchez-Nazario *et al.* (2003) demonstrated that toy chewing, along with Pb levels in window sills and soil eating habits, were significant predictors of blood Pb levels in children. Toy chewing may be a route of dust ingestion as well as absorption of Pb from the toy. Children may be exposed to Pb in other consumer products including plastic window blinds, Pb core candle wicks, or backpacks (Sanborn *et al.* 2002).

Renovating, repairing, or painting a pre-1978 building can release particles of Pb-based paint and is associated with increases in blood Pb levels in children and adults who live in the home (CDC 2009a, 2011a). Thorough cleaning after completion of remodeling is effective in removing a majority of the Pb dust from a renovated residence (Yiin *et al.* 2004). Proper maintenance of housing by people trained in lead-safe practices, focusing on residential complexes with previous cases of elevated blood Pb levels can prevent future Pb exposures (CDC 2005b). Construction and painting also contribute to occupational Pb exposures (CDC 2011a). Contractors engaged in renovation or remodeling must be certified through the EPA Lead-Safe Certification Program and employ safe work practices to reduce Pb exposures to their clients, employees, and themselves. Additional certification at the state or Federal level is required for abatement to permanently eliminate lead-based paint hazards from a home (**http://www.epa.gov/lead/pubs/traincert.htm**).

Some hobbies or recreational activities are potential sources of Pb exposure (Sanborn *et al.* 2002). Hobbies include furniture refinishing, jewelry making, creating stained glass, print-

making, enameling copper, casting bronze or lead figurines, leaded glass blowing, working with Pb solder on electronics, and using Pb containing paints or pottery glazes (CDC 2010). Fishing and hunting can contribute to Pb exposure when making fishing weights, casting ammunition, or eating animals contaminated with Pb after ingesting Pb shot or fishing weights (CDC 2010). Air Pb levels in indoor firing ranges were significantly higher in ranges that used powder charges (660µg/m^3) than those that used air guns (4.6µg/m^3) or archery ranges (0.11µg/m^3), and blood Pb levels were significantly higher for marksmen using powder charges during the indoor shooting season (Svensson *et al.* 1992). Pb exposures from these hobbies can be significant: a potter and her family experienced elevated Pb levels from Pb glazes used in a home studio (48µg/dL for the potter, 54µg/dL for her daughter, and 20µg/dL for her husband), and a man whose hobbies included melting Pb weights to make figurines and shooting firearms at an indoor firing range had a blood Pb level of 39µg/dL (Fischbein *et al.* 1992).

Occupational exposures to Pb occur in more than 100 industries where Pb or Pb-containing materials are used or disturbed by workers (CDC 2010). Approximately 95% of all elevated blood Pb levels reported in adults in the United States are work-related (CDC 2011a). The prevalence rate of workers with blood Pb levels over 25µg/dL decreased by more than 50% from 1994 to 2009 (from 14 to 6.3 per 100,000 adult workers), and in 2009 the Adult Blood Lead Epidemiology and Surveillance (ABLES) program lowered their definition for elevated blood Pb level from 25 to 10µg/dL due to increased concern over health risks from lower blood Pb levels (ABLES 2009). The lowest blood Pb level required to be reported under state laws varies by state; however, of the 10 states that collected all test levels in 2004, 32% of women with blood Pb >5µg/dL reported occupational exposures, mostly in manufacturing (CDC 2010). Occupational sources of Pb can also expose workers' families as Pb dust travels home on clothes and in vehicles (Hipkins *et al.* 2004). Living near Pb mining, smelting, and manufacturing sites may expose the surrounding community to low Pb levels, particularly in countries without environmental regulations or monitoring programs (Benin *et al.* 1999). These populations have been the subject of many older studies of health effects associated with Pb exposure and continue to be a source of study subjects with higher exposure levels (e.g., a recent study of birth outcomes associated with damage to a pollution-control device at a Pb smelter plant Berkowitz *et al.* 2006).

Due to the focus of this evaluation on blood Pb levels below 10µg/dL, studies with mean blood Pb levels >15µg/dL were not included in this evaluation except as specified in **Section 8.0 Reproductive / Developmental Effects** (e.g., Kromhout *et al.* 1985; Lockett and Arbuckle 1987). Stratified analyses of only subjects above and below 10µg/dL have indicated that associations with some health effects can be stronger at lower exposure levels (e.g., Pb- related intellectual deficits Lanphear *et al.* 2005). Excluded occupational studies were mostly older publications on workers with mean blood Pb levels >10µg/dL or in workers without occupational monitoring programs. Even with the 10µg/dL ABLES definition of elevated blood Pb, Pb-exposed workers can have higher blood levels than the general population and a higher lifetime burden of Pb from long term exposures.

3.4 Modifiers of Pb Exposure

Individual-level differences in exposure and biology impact the amount of Pb that reaches a target tissue to impact health. These differences may influence contact with environmental Pb, Pb metabolism, and remobilization of Pb stores. Modifiers of Pb exposure include age, life stage, gender, diet, socioeconomic status, immigrant status, and genetic variants. These factors are often correlated with one another as well.

Blood Pb levels increase with age from bone Pb stores that accumulate over time, as previously discussed in **Section 3.2 Biomarkers of Pb Exposure**. Prior to bans on Pb in paint, solder, and gasoline, environmental Pb levels in the United States were higher, so older adults accumulated more Pb as children than children do today. Studies have suggested that the aging process contributes to Pb exposure as bone begins to deteriorate, particularly if coupled with osteoporosis (Silbergeld *et al.* 1988). A study of adults in New York found that age was not a risk factor for higher blood Pb levels (10µg/dL or more) (Gelberg and Fletcher 2010); however blood Pb levels <10µg/dL were not reported. Recent NHANES data support a positive correlation between blood Pb and age in older children and adults with generally low blood Pb levels (well below 10µg/dL, see **Figure 3.3**) (CDC 2005a). Young children are an exception to this age trend and have higher blood Pb levels than infants and older children (CDC 2007).

Young children show marked increases in blood Pb levels after birth with a peak around age 2 (Rothenberg *et al.* 1999b). Initially, maternal sources of Pb could contribute to a child's exposure levels. Mothers' blood Pb levels at delivery are highly correlated with cord blood Pb levels, with cord blood levels slightly lower (Graziano *et al.* 1990; Rothenberg *et al.* 1999b). The CDC concluded that *in utero* exposure risks to children are greatest if mothers had a significant past Pb exposure (CDC 2010). Maternal blood and milk Pb levels are correlated as well, but the efficiency of Pb transfer from blood to milk varies at low levels, and Koyashiki et al. concluded that there are no established health risks from breast milk (2010). Current CDC guidelines are to continue breastfeeding up to high blood Pb levels (40µg/dL blood Pb levels in the mother) (CDC 2010). A study in mice showed that gestational and lactational Pb exposure from the mother increases Pb levels in the offspring, with declining blood Pb levels after weaning (Snyder *et al.* 2000). There is some evidence that Pb from dietary sources is more readily absorbed and retained in young children and infants than in adults (Ziegler *et al.* 1978). Young children are also exposed to environmental Pb due to normal mouthing behaviors as discussed in **Section 3.3 Sources of Pb**.

On average adult men have higher levels of Pb in blood and bone than women, and men are much more likely to be exposed to occupational sources of Pb. However, women typically go through more stages of life where bone stores are mobilized into circulating Pb. Women are at risk from bone mobilization during pregnancy, menopause, and due to osteoporosis (Manton *et al.* 2003; Silbergeld *et al.* 1988). Blood Pb levels in pregnant women are generally low in the United States (NHANES geometric mean <5µg/dL) and do not vary by the age of the mother (Jones *et al.* 2010). Pregnancy-associated elevations in blood Pb have been demonstrated in a number of case studies (e.g., Rothenberg *et al.* 1992; Shannon 2003), but the overall pattern of

blood Pb throughout pregnancy appears to be complex (Hertz-Picciotto *et al.* 2000; Schell *et al.* 2000). Blood Pb levels follow a U-shaped curve during pregnancy, decreasing from weeks 12-20 and then increasing linearly over the second half of pregnancy (Rothenberg *et al.* 1994). Overall blood Pb levels decrease during subsequent pregnancies, so the first pregnancies pose the most risk of Pb toxicity, particularly if the mother had significant past Pb exposures (CDC 2010; Manton *et al.* 2003).

Nutritional deficiencies in calcium, iron, and zinc were associated with increased Pb levels at age six months, and iron deficiency continued to be associated with Pb at the age of 12 months (Schell *et al.* 2004). Low iron intake may contribute by increasing Pb absorption in these infants with a mean 12-month blood Pb level of 5.1µg/dL (Schell *et al.* 2004). In older people, calcium deficiency can increase bone turnover and circulating Pb levels (CDC 2010). Pb absorption is higher when there is less food in the digestive tract making dietary habits and gastric emptying rates another source of individual variation in the body burden of Pb (James *et al.* 1985; Maddaloni *et al.* 1998).

Low socioeconomic status (SES) is associated with higher blood Pb levels (Schnaas *et al.* 2004; Wibowo *et al.* 1986). People with low SES may be exposed to a collection of risk factors including living in older, deteriorated housing with Pb in paint, household dust, pipes, or urban air; consuming diets lower in nutrients and calories; playing with potentially contaminated inexpensive toys; being employed in jobs with occupational Pb exposure; and other environmental hazards (Jones *et al.* 2009; Sexton 1997; Strike and Steptoe 2004). The best strategy for preventing new Pb exposures in housing is to remove the Pb paint and dust, but authors such as Wakefield et al. (2002) have noted that lead abatement can cost over $10,000 per home and they suggest that this cost may result in remediation of less than 0.1% of seriously dangerous homes per year. Care has to be taken during the remediation and training is required, as discussed earlier, because, general repair and renovation can be associated with increased Pb exposure and higher blood Pb levels in building occupants and workers performing the repairs (CDC 2009a, 2011a). Pb is associated with an increased cardiovascular response to stress (Gump *et al.* 2005), and low SES was a moderator of this response in the Oswego Children's Study (Gump *et al.* 2007). For some associations of Pb with health outcomes (e.g., for developmental neurotoxicity Bellinger 2008), higher Pb levels in people with low SES make SES a relevant confounder for researchers to consider (Bellinger 2008; Factor-Litvak *et al.* 1999).

Many immigrants face SES-related exposure risks, but they may have additional risk factors as well. If their home country has relatively high Pb exposure levels, immigrants carry a larger body burden of Pb (CDC 2010). Exposure to leaded gasoline emissions, as estimated from time spent in Mexico City, was a major source of cumulative Pb exposure, in a study of postpartum women in Mexico (Brown *et al.* 2000). In a study of pregnant women, a Pb-related increase in blood pressure was only seen in immigrants, predominantly from Latin America, even without markedly higher blood Pb levels than non-immigrants (Rothenberg *et al.* 1999a). In some cultures, pica during pregnancy is common and accepted (CDC 2010). In a study of pregnant women in New York, pica was the most frequently reported source of Pb exposure (13 women,

39% of those with levels >20µg/dL) (Klitzman *et al.* 2002). Immigrant status could increase exposure to Pb contaminated products including alternative remedies, imported cosmetics or food items, or Pb-glazed pottery for cooking or food storage (CDC 2010). US women using herbal supplements had higher blood Pb levels; particularly in those using St. John's wort, Ayurvedic, or traditional Chinese medicinal herbs (Buettner *et al.* 2009).

Biological variation in Pb absorption and metabolism rates can be partially explained by genetic variation. The relationship between Pb exposure and a particular health effect may be modified by the presence of a single nucleotide polymorphism (SNP) or other genetic variant. When studying genetic risk factors in observational studies, selection for the study is independent of genotype and unknown to the subject, minimizing sources of bias that may confound other factors. If specific genetic variants are found to increase or decrease the association of Pb with a health outcome, there is a stronger biological basis for that relationship, and the gene function may give an indication of the mechanism of action. Genes studied for variations in Pb metabolism include hemochromatosis (*HFE*) and aminolevulinate dehydratase (*ALAD*) and specific study details are presented in the appendices for each chapter.

Interactions between many of the previously discussed factors make it difficult to separate the increases in risk from each individual factor. While many Pb exposure reduction measures have decreased blood Pb levels in the US population, economically disadvantaged young children in older housing or pregnant immigrants using contaminated products are still at risk for significant Pb exposures.

3.5 Summary

While Pb can be measured in a variety of human tissues, whole blood Pb is the most common measure used in both research and clinical settings. Blood Pb levels fluctuate and represent both current exogenous exposures and endogenous sources of Pb, primarily stored in bone. For studies of chronic health effects, bone Pb is a better measure of the cumulative body burden of Pb and may show more consistent associations with long term health outcomes. Pb continues to be used in industrial work processes and manufactured products in the United States and worldwide, is persistent in the environment, and humans are exposed to Pb via water, air, soil, food, and consumer products. Several Pb reduction efforts have significantly reduced exposure levels over the last 30 years and blood Pb levels have dropped considerably in the United States. Pb exposure levels vary greatly by age, life stage, gender, and socioeconomic level; and even at low levels with blood Pb <10µg/dL there are health risks. The other chapters of this document outline the evidence for specific health effects from blood Pb levels below 10µg/dL. A discussion of Pb exposures in potentially susceptible populations for specific health effects is included in individual chapters.

4.0 NEUROLOGICAL EFFECTS

4.1 Conclusions:

The NTP concludes that there is *sufficient* evidence that blood Pb levels <10µg/dL are associated with adverse neurological effects in children and and *limited* evidence in adults. A major strength of the evidence for effects of low-level Pb on neurological outcomes is the consistency of results for an adverse effect of blood Pb<10µg/dL across multiple indices of neurological effects (e.g., cognition, behavior and sensory function), through multiple populations, a wide age range from early childhood to older adults, and from studies with substantial methodological heterogeneity.

Unlike the dataset for most other health outcomes, there are a number of prospective studies of neurological effects that include prenatal exposure metrics (either maternal blood or cord blood). These prospective studies provide *limited* evidence that prenatal exposure to blood Pb levels <5µg/dL are associated with decreases in measures of general and specific cognitive function evaluated in children. There is also *limited* evidence that prenatal exposure to blood Pb levels <10µg/dL are associated with decreased IQ, increased incidence of ADHD and antisocial behavior problems, and decreased hearing measured in children. Conclusions on effects of prenatal exposure for outcomes evaluated as children are complicated by the high degree of correlation in childhood blood Pb levels over time and as described below, blood Pb levels during childhood are also associated with these outcomes.

In children, there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with broad-based and specific indices of reduced cognitive function and an increase in ADHD diagnosis and antisocial problem behaviors. Lower levels of academic achievement determined by class rank and achievement tests have been reported in multiple prospective and cross-sectional studies of children with blood Pb <5µg/dL. A negative association between blood Pb at levels <5µg/dL and specific measures of cognitive function have been demonstrated in prospective and cross-sectional studies using a wide range of tests for assessment. Increased diagnosis of ADHD and behavioral problems are consistently reported in studies with mean blood Pb levels <5µg/dL. There is *sufficient* evidence that blood Pb levels <10µg/dL in children are associated with decreases in full-scale IQ (FSIQ) score and decreased auditory acuity. There is consistent evidence that blood Pb is associated with decreased IQ across multiple prospective studies of children and in well accepted pooled analyses (Lanphear *et al.* 2005) demonstrating effects at blood Pb levels <10µg/dL. Multiple cross-sectional studies reported hearing loss indicated by higher hearing thresholds and increased latency of brainstem auditory evoked potentials in children with blood Pb levels<10µg/dL.

In adults, there is *limited* evidence that blood Pb levels <10µg/dL are associated with psychiatric outcomes including anxiety and depression, decreased auditory function, decreases in specific measures of cognitive function in older adults, and neurodegenerative diseases including ALS and essential tremor. As with other studies of health effects of Pb in adults, prospective studies in a population for which the data demonstrated that blood Pb levels remained consistently

below 10µg/dL from birth until measurement of the outcome of interest would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL. There are more consistent associations between bone Pb than blood Pb and decreases in cognitive function in older adults, suggesting a role for cumulative Pb exposure.

4.2 How conclusions were reached:

Conclusions in the NTP evaluation of Pb-related neurological effects in humans associated with low-level Pb are derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10µg/dL. The NTP conclusions are based on the evidence from human studies with blood Pb levels of ≤10µg/dL and with data reflecting exposure levels up to 15µg/dL also considered so that effects at and around 10µg/dL were not excluded from the evaluation.

There is a relatively large database of human studies for a wide range of neurological effects (see **Table 4.1**) of low-level Pb and therefore, the document makes limited use of the data from laboratory animals to support the human evidence. Major endpoints considered as potential indicators of neurological effects of Pb are listed and briefly described in **Section 4.2.1**. This document is not a review of neurotoxicity and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP conclusions are discussed in detail in **Section 4.3 Evidence for Pb-related Effects on Neurological Outcomes**. The discussion of

Table 4.1: Major Neurological effects considered

Effect	Description
Cognitive Function:	
IQ – an index of global cognitive function	**Full-scale IQ** (**FSIQ**); verbal IQ (**VIQ**); performance IQ (**PIQ**) Evaluated with a variety of tests, e.g., Stanford-Binet Intelligence Scale or Wechsler Intelligence Scales for Children (WISC). Note, WISC is a normative measure for assessing intelligence in children allowing cross-study comparison (e.g.Lanphear *et al.* 2005)
Academic Achievement	Academic performance is measured with a variety of tests such as Boston Teachers Questionnaire (BTQ), Wide Range Achievement Test-Revised (WRAT-R) and more recently with end-of-grade testing and Standard Assessment Tests (SATs)
General and specific cognitive abilities	Numerous tests of cognitive function including general measures such as the Bayley mental development index (MDI); McCathy Scales of Children's Abilities including the General Cognitive Index(GCI); or specific measures such as individual subsets of the WISC such as Block Design or Digit Span
Behavior and Psychiatric outcomes	
Behavior	Numerous measures of behavioral outcome evaluated with tests such as Behavioral Assessment System for Children (BASC) **ADHD** - attention deficit hyperactivity disorder measured with a variety of evaluation tools e.g., Conner's ADHD Diagnostic and Statistical Manual of Mental Disorders (DSM) **Conduct or problem behavior** related outcomes measured with a variety of evaluation tools e.g., Teacher Report Form-Delinquent Behavior, self-reported delinquent behavior (SRDB)
Psychiatric outcomes	Mood disorders are diagnosed with various tests such as the Child Behavior Checklist (CBC)or Brief Symptom Inventory (BSI)
Neurodegeneration	
Various diseases	Amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Essential Tremor (ET), Parkinson's disease
Sensory function	
Audio	Several measures of audial function e.g., higher hearing thresholds and altered brainstem auditory evoked potentials (BAEP)
Vision	Several measures of visual function e.g., altered visual evoked potentials (VEP) and electroreinographic (ERG) testing

each neurological effect begins with a statement of the NTP's conclusion that the specific effect is associated with a blood Pb level <10µg/dL or <5µg/dL and the age group in which it is identified (childhood or adulthood) as well as the timing of exposure associated with the effect (prenatal, childhood, or concurrent), when available. Although the information necessary to support the NTP's conclusions is presented in **Section 4.3**, the complete dataset of human studies considered for evaluation of neurological effects with low-level Pb is included in the Neural Appendix and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 4.2.2** below.

4.2.1 Principal Measures of Neurological Effects

Table 4.1 lists a number of key neurological endpoints evaluated in epidemiological studies on the effects of Pb exposure and identifies representative tests that have been used to evaluate major neurological effects of Pb. A list or review of the full range of tests and tools used to evaluate neurocognitive, neurobehavioral, psychiatric, and neurophysiological outcomes is beyond the scope of this evaluation. The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 4.3** below.

4.2.2 Principal conclusions from the 2006 EPA and 2007 ATSDR Pb documents:

Table 4.2: Main conclusion for neurological effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead
"….effects on neurobehavior in children have been observed with remarkable consistency across numerous studies of various designs, populations, and developmental assessment protocols. The negative impacts of Pb on neurocognitive ability and other neurobehavioral outcomes persist in most recent studies even after adjustment for numerous confounding factors, including social class, quality of caregiving, and parental intelligence" (EPA, 2006 pg 6-269)
"…the preponderance of the evidence indicates that lead exposure is associated with decrements in cognitive function. Meta-analyses conducted on cross-sectional studies or a combination of cross-sectional and prospective studies suggest that an IQ decline of 1–5 points is associated with an increase in PbB of 10 µg/dL. Most importantly, no threshold for the effects of lead on IQ has been identified… "(ATSDR, 2007 pg 25)

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both concluded that negative effects of Pb on neurocognitive ability and neurobehavioral outcomes in children are observed across numerous studies at blood Pb levels <10µg/dL even after adjusting for confounding factors (see **Table 4.2**). The Lanphear *et al.* (2005) pooled analysis is cited by both Agencies as supporting evidence for a decline up to 6 FSIQ points for an increase in blood Pb from 1 to 10 µg/dL in children. The 2006 EPA AQCD for Lead and the 2005 CDC statement on Preventing Lead Poisoning in Young Children (CDC 2005) highlights the evidence for a supralinear dose-response relationship for some neuro-developmental outcomes (particularly IQ) and a steeper dose-response curve at lower Pb levels below 10µg/dL. The EPA 2006 AQCD for Lead also identifies recent evidence that Pb-associated neurocognitive deficits are associated with decreased academic achievement and notes that

negative effects of Pb on attention may contribute to achievement or delinquent behavior in children. The EPA 2006 AQCD for Lead concludes that the negative impacts of Pb on neurocognition and behavior persist into young adulthood and that there is clear evidence that blood Pb levels in children of 5-10μg/dL (and possibly lower) are associated with these negative effects. In adults, both ATSDR and EPA noted that chronic occupational Pb exposure is associated with decreased nerve conduction velocity and postural balance abnormalities. The EPA 2006 AQCD for Lead identified ≥14μg/dL blood Pb level as a possible threshold for these effects as well as visuomotor and memory impairment, and effects on the visual and auditory systems (prolonged visual evoked and brainstem auditory evoked potentials). The EPA 2006 AQCD for Lead characterized the evidence for Pb-associated impaired cognitive performance in adults as mixed, although bone Pb (and therefore long term cumulative exposure) was associated with decreased cognitive performance. The EPA 2006 AQCD for Lead stated that four studies reported that past occupational exposure to Pb increased the risk of developing ALS and motor neuron disease and two studies reported that essential tremor was associated with low blood Pb levels (mean 3μg/dL). EPA is in the process of revising the AQCD for Lead, and the conclusions of the external draft (U.S. EPA 2011) are largely in line with the 2006 AQCD for Lead plus additional review of the evidence for attention deficit hyperactivity disorder (ADHD).

The NTP considered the conclusions and data summaries from the EPA and ATSDR documents. In general, the NTP accepted the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints.

4.3 Evidence for Pb-related Effects on Neurological Outcomes

4.3.1 Cognitive Function

There is *sufficient* evidence that blood Pb levels <5μg/dL are associated with decreased cognitive function in children. There are many tests and measures used to evaluate cognitive function and blood Pb levels are associated with decreases in broad-based measures in children such as academic achievement or FSIQ, as well as specific cognitive measures evaluated in all age groups from young children to older adults. No clear and specific pattern of Pb-related decrements in specific cognitive abilities has been identified, although decreased performance on individual domains for attention, executive function, language, learning and memory, and visual-spatial processing have been reported (ATSDR 2007; U.S. EPA 2006). Generally, the lack of clear differences in sensitivity and specificity for individual domains is attributed in part to difficulty discriminating focused effects because test performance covers multiple neurobehavioral processes (U.S. EPA 2007).

The discussion of cognitive function below is divided into three sections focused on:
(1) academic achievement – a practical and perhaps more objective measure of cognitive function in children that may relate to achievement in life through education-based achievement measures, (2) IQ in children, and (3) other general and specific measures of cognitive abilities in children and adults.

Academic Achievement

There is *sufficient* evidence that blood Pb levels <5µg/dL are associated with decreases in various measures of academic achievement in children aged 6-18 (see Appendix A: Neurological Effects for full list of studies). A negative association between blood Pb level and performance in tests of academic performance, class rank, or end of grade testing has been reported in multiple prospective and cross-sectional studies involving children with blood Pb levels from 2 to 10µg/dL from populations in North America, Europe, and Africa. Studies demonstrated that early childhood blood Pb (9-36 months) or tooth dentin levels (6-8 years of age) are associated with decreased academic achievement measured as children from age 10-18. Data from cross-sectional studies also support a negative effect of concurrent blood Pb levels (between 5 and 10µg/dL) on academic achievement. However, there is *inadequate* evidence that prenatal blood Pb levels <10µg/dL are associated with academic achievement in children because of a lack of academic performance studies that include Pb exposure data from prenatal time points.

A number of studies have used academic achievement as an indicator of effects of Pb on cognitive function in children. Many of the earlier studies of academic achievement used tooth dentin Pb as the measure of Pb exposure and compared measures of educational attainment in children with earlier blood Pb or dentin Pb levels. For example, higher tooth dentin Pb levels at ages 6-8 were associated with: lower class standing in 132 children in Massachusetts at age 18 in Needleman *et al.* (1990); reading and spelling difficulties in 8-year-old girls (n=1923 boys and girls) assessed with the Boston Teachers Questionnaire (Leviton *et al.* 1993); and various measures of achievement assessed from ages 8 to 18 in 1265 children from the Christchurch Health and Development Study cohort including number of school certificate passes, Burt Word Reading test assessed at ages 8, 12, and 18 (Fergusson *et al.* 1988; Fergusson *et al.* 1993, 1997). Bellinger *et al.* (1992) reported that blood Pb levels (mean 6.5µg/dL) at 2 years of age, but not with cord blood Pb or blood Pb levels at 6, 12, 18, or 57 months of age, were significantly associated with lower scores in the Kaufman Test of Educational Achievement (KTEA) in 148 children aged 10 from the Boston Lead Study. In general, the data support a persistence of the negative effect on cognitive achievement that results in reduced educational attainment. However, at least one study reported that tooth dentin Pb levels were not associated with achievement, e.g., Rabinowitz *et al.* (1992) stated that tooth Pb levels were not associated with scores on the Boston Teachers Questionnaire in a study of 493 Taiwanese children.

More recent studies have demonstrated a negative effect of concurrent or early childhood blood Pb levels on academic achievement at blood Pb levels <10µg/dL and down to 2µg/dL. In a cross-sectional study of 4853 children 6-16 years of age from the NHANES III dataset, Lanphear *et al.* (2000) demonstrated that concurrent blood Pb levels <5 µg/dL (geometric mean 1.9µg/dL) were associated with decrements in achievement measured by the Wide Range Achievement Test (WRAT-Revised). As with other cross-sectional studies of Pb, an important limitation is that blood Pb during early childhood is likely to have been higher than blood Pb measured during the study; and therefore, blood Pb may have been above 10µg/dL at earlier time points for children in this study. In a cross-sectional analysis of 511 US children between 6 and 10 years of age, concurrent blood Pb ≥5µg/dL was associated with lower scores on the Wechsler Individual Achievement Test (Surkan *et al.* 2007). In a cross-sectional study of 533

girls in Saudi Arabia between 6 and 12 years of age, Al-Saleh *et al.* (2001) reported that class rank percentile was negatively associated with concurrent blood Pb levels (mean 8µg/dL). Wang *et al.* (2002) found a similar negative relationship between concurrent blood Pb level (mean =5.5µg/dL) and class ranking of 934 Taiwanese children aged 9 for individual subject areas (e.g., Chinese and natural sciences). In subgroup analysis, Min *et al.* (2009) reported that blood Pb <5µg/dL measured at 4 years of age was significantly associated with decreased school reading scores (Woodcock Johnson-III Tests of Achievement) at age 9 and 11 in a prospective study of 278 inner-city children from Cleveland, OH. Chandramouli *et al.* (2009) demonstrated that educational performance on SATs at 7-8 years of age was negatively associated with blood Pb levels ≥5µg/dL at 30 months of age in a prospective study of 582 children in the United Kingdom. Surkan *et al.* (2007) reported that blood Pb of 5-10µg/dL was associated with decreased performance in the Wechsler Individual Achievement Test in a study of 6-11-year-old children in Maine and Massachusetts. Miranda *et al.* (2007) reported that blood Pb levels down to 2µg/dL (collected as part of Pb screening programs when the children were below the age of 5) were negatively related to test performance in both reading and mathematics in a study of 8,603 children in the 4th grade (9-10 year olds) from four counties in North Carolina. In an expanded study of over 57,000 children in the 4th grade from across North Carolina, and screened for blood Pb between 9 months and 3 years of age, Miranda *et al.* (2009) demonstrated a similar effect of blood Pb levels down to 2µg/dL on end of grade reading scores compared to the reference group with blood Pb levels of 1µg/dL.

No studies were located that evaluated maternal Pb levels and educational achievement; however, the Bellinger *et al.* (1992) study reported that cord blood Pb levels (29% of study group ≥10µg/dL) were not significantly associated with lower scores in the Kaufman Test of Educational Achievement (KTEA) in 148 children aged 10 from the Boston Lead Study.

Confounding variables were considered in all of the studies listed above to one degree or another, but the Miranda *et al.* (2007; 2009), Chandramouli *et al.* (2009), and Lanphear *et al.* (2000) studies included a large number of confounders such as socioeconomic variables, sex, race/ethnicity, age of blood Pb measurement, parental education and tobacco exposure and demonstrated that the effects on achievement were independent of known confounders. The Miranda *et al.* (2009) study found evidence of differential effects in students with lower scores. Miranda *et al.* (2009) reported that children with the lowest test scores had a larger negative effect of blood Pb in lowering the test score; similar results of a greater impact in children with lower scores were found for a negative effect of lower SES and lower parental education suggesting that Pb and multiple confounders all have a greater impact in individuals that already have lower achievement. The Surkan *et al.* (2007) and Bellinger *et al.* (1992) studies reported a negative effect of Pb on academic achievement while controlling for the child's IQ, suggesting that IQ and academic performance may serve as somewhat independent measures of cognitive function.

Summary of support for conclusions

Animal data support a Pb-associated decrease in neurobehavioral tests of learning including fixed interval operant conditioning at blood Pb levels ≥11µg/dL (see ATSDR 2007; U.S. EPA 2006

for recent reviews of the animal data). The human data include multiple prospective and cross-sectional studies supporting a Pb-associated decrease in educational attainment at blood Pb levels from 2 to 10µg/dL. The conclusion of *sufficient* evidence for decreased academic achievement in children aged 6-18 with blood Pb levels <5µg/dL measured in early childhood through age 18 is based on the consistency of effects on several measures of academic achievement in multiple studies. The Bellinger *et al.* (1992) study includes multiple blood Pb measurements and only found a negative effect of blood Pb measured at 2 years of age with later life academic performance. Multiple studies (e.g., Chandramouli *et al.* 2009; Miranda *et al.* 2007; Miranda *et al.* 2009) reported that early childhood Pb exposure is associated with later performance. However, clear evidence that early childhood Pb exposure is associated with academic performance at later ages is complicated by the high degree of correlation in childhood blood Pb levels over time (e.g., see Dietrich *et al.* 1993a; Lanphear *et al.* 2005). Current evidence can be used to support the importance of both early-life exposure and current blood Pb levels. The conclusion of *inadequate* evidence that prenatal blood Pb levels <10µg/dL are associated with academic achievement in children is based on the lack of studies of academic performance in children with Pb exposure data from prenatal time points. The NTP's conclusions for *sufficient* evidence that decreased academic achievement in children aged 6-18 is associated with Pb levels <5µg/dL, extends the conclusion from EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead which were limited to blood Pb levels <10µg/dL; however, the EPA's 2011 draft (U.S. EPA 2011) currently supports a lower blood Pb level.

IQ

There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with decreases in full-scale IQ (FSIQ), a global measure of cognitive ability in children aged 4-13 (see Appendix A: Neurological Effects for full list of studies). Multiple prospective studies demonstrated that blood Pb levels <10µg/dL during early childhood are associated with decreased IQ measured in children age 6 to 13 from populations in North America, Australia, Europe and Asia by a range of different tests to assess IQ. Many recent studies have used the Wechsler Intelligence Scales for Children (WISC) and adopted similar protocols in an effort to facilitate cross-study comparison and greater generalization of the findings. The most recently published pooled analysis of IQ data, Lanphear *et al.* (2005), took advantage of the similarity in study design of seven prospective studies and concluded that blood Pb levels <10µg/dL in children are associated with intellectual deficits. Data from cross-sectional studies also support an association between concurrent blood Pb levels <10µg/dL and decreased IQ in children up to age 13. The evidence is mixed for an association between maternal or cord blood Pb and decreased IQ in children evaluated at a later age. Therefore, there is *limited* evidence that prenatal blood Pb levels <10µg/dL are associated with decreased IQ in children.

A number of prospective studies have reported an association between blood Pb <10µg/dL and decreased IQ score in children from ages 4-13. The decrease in IQ has been reported in cohorts for whom the blood Pb level remained below 10µg/dL from birth to evaluation. For example, In a prospective study of 148 children with serial blood Pb measurements from birth through 10 years of age, Bellinger *et al.* (reanalyzed in Bellinger and Needleman 2003; 1992) demonstrated

that blood Pb levels at the age of 2 (mean 6.5µg/dL), but not other ages, were significantly associated with decreases in FSIQ and verbal-IQ (VIQ) assessed by the WISC-R at age 10 in a cohort from the Brigham and Women's Hospital; the association with performance-IQ (PIQ) was not significant (p=0.091). Min *et al.* (2009) demonstrated that blood Pb measured at 4 years of age (mean 7µg/dL) was significantly associated with decreased FSIQ by the Wechsler Preschool and Primary Scales of Intelligence (WPPSI-R) at 4 years of age and FSIQ by the full WISC-IV at ages 9 and 11 in a prospective study of 278 inner-city children from Cleveland, OH. However, many of the early studies were in children with blood Pb levels >10µg/dL at some stage prior to administering the test to evaluate IQ. For example, Baghurst *et al.* (1992) reported that blood Pb levels during early childhood up to age 4 (but not maternal blood Pb, cord blood Pb, or average through age 7) were significantly associated with decreased FSIQ and VIQ scores evaluated at age 7 by the WISC-R years in 494 children in Port Pirie Australia; however, mean blood Pb levels in this cohort were above 10µg/dL after birth. A similar association between blood Pb and decreased FSIQ has been demonstrated in a number of prospective studies with cumulative mean blood Pb levels >10µg/dL that evaluated IQ in children at 5-13 years of age (Canfield *et al.* 2003a; Dietrich *et al.* 1993b; Factor-Litvak *et al.* 1999; Jusko *et al.* 2008; Tong *et al.* 1996; Wasserman *et al.* 2003; Wasserman *et al.* 1997).

An association between concurrent blood Pb <10µg/dL and decreased IQ has been reported in several studies. Chiodo *et al.* (2007; 2004) evaluated a range of neurodevelopmental endpoints in two publications covering between 240 and 500 African American inner-city children aged 7-9 with concurrent blood Pb levels (mean 5.4µg/dL); blood Pb levels were related to decreased FSIQ, PIQ, and VIQ as well as behavioral endpoints evaluated with the WISC-III. When dichotomized by cut points of 10, 7.5, 5 and 3µg/dL blood Pb, the study supported significant effects on IQ at blood Pb levels of ≥5µg/dL with some support for effects at ≥3µg/dL. In a cross-sectional study, Kim *et al.* (2009) reported that concurrent blood Pb (mean 1.7µg/dL) in a study of 261 children in Korea aged 8-11 was significantly associated with reduced FSIQ and VIQ by the Korean Educational Development Institute – Wechsler Intelligence Scales for Children test. This study represents the cohort with the lowest mean blood Pb level associated with decreased IQ; however, the cross-sectional nature of the Kim *et al.* (2009) does not provide information to verify that blood Pb levels were <5 or <10µg/dL from birth to age at which the test was administered.

In a study of 253 children from the Cincinnati Lead Study, Dietrich *et al.* (1993b) reported that decreases in FSIQ and PIQ by WISC-R were significantly associated with concurrent blood Pb levels at 6 years of age and blood Pb for the year prior (down to age 3 for PIQ); however, IQ was not associated with maternal Pb, cord blood Pb, or blood Pb levels during early childhood. Hornung *et al.* (2009) performed an analysis to determine the age of greatest susceptibility to blood Pb in contributing to lower IQ scores evaluated with the WISC-R in a combined cohort from the Cincinnati and Rochester Lead studies (n=397 total) with peak blood Pb mean =13.6µg/dL and concurrent blood Pb at 6 years of age = 6µg/dL. Concurrent blood Pb (age 6; β=-3.48;p<0.001) and the prior year (age 5; β=-4.39; p<0.001) had the strongest effect on IQ evaluated at age 6 compared to blood Pb at earlier ages (e.g., age 1; β=-0.08;p=0.934). Note that peak childhood blood Pb in the Cincinnati and Rochester cohorts was above 10µg/dL;

therefore, it is not clear that the observed decreases in IQ reported in Dietrich et al. (1993b) and Hornung et al. (2009) are strictly associated with a blood Pb level <10µg/dL.

No clear evidence was located that maternal or cord blood Pb levels <10µg/dL are associated with decreased IQ in children. Many studies, such as Baghurst *et al.* (1992), Bellinger *et al.* (1992), and Dietrich *et al.* (1993b) described earlier, did not find a significant association with prenatal blood Pb levels in cohorts for which prenatal blood Pb was <10µg/dL. In a prospective study that examined the relationship between maternal blood Pb during early and late pregnancy and FSIQ by the WISC-R in children, Schnaas *et al.* (2006) reported that maternal blood Pb levels at 28-36 weeks of pregnancy (mean 7.8µg/dL) were related to reduced IQ in children aged 6-10. However, as in other studies, the mean blood Pb in this cohort was above 10µg/dL during early childhood.

The evidence for a Pb-related decrease in IQ has been the subject of four key meta-analyses (Lanphear *et al.* 2005; Needleman and Gatsonis 1990; Pocock *et al.* 1994; Schwartz 1994) and was extensively reviewed in the 2006 EPA AQCD for Lead (U.S. EPA 2006) and ATSDR Toxicological Profile for Lead (ATSDR 2007). These analyses provide strong support that blood Pb and tooth Pb are significantly associated with decreases in IQ in children; however, many of the cross-sectional and prospective studies included in these analyses are based on cohorts with blood Pb levels >10µg/dL at some age from birth to evaluation of IQ. The Schwartz *et al.* (1994) and Lanphear (2005) meta-analyses support effects at blood Pb levels <10µg/dL, although these analyses face challenges of confounding and various tests used to assess IQ across studies. However, the 2005 pooled analysis (Lanphear *et al.* 2005) reduced these confounders by pooling data from seven prospective studies that used similar test protocols (e.g., relying principally on the WISC) and included 1333 children from multiple countries. The analyses supported an association between blood Pb levels <10µg/dL and decreased IQ and the authors concluded that blood Pb levels <10µg/dL (and specifically <7.5µg/dL) in children are associated with intellectual deficits. Lanphear (2005) reported significant decreases in IQ in analyses restricted to children with a maximum blood Pb level <10µg/dL. The authors also attempted to characterize the shape of the dose-response between blood Pb and IQ (discussed further in following discussion).

Although some earlier studies of Pb and cognitive function did not include adjustment for maternal IQ or other confounders, these studies were also generally in children with blood Pb levels >10µg/dL. More recent studies of the relationship between blood Pb and IQ in children with blood Pb levels <10µg/dL considered a wide range of potential confounders. For example, the Lanphear *et al.* (2005) pooled analysis included maternal IQ, maternal education, Home Observation for Measurement of the Environment (HOME) score, and birth weight in the final model; however, prenatal smoking, prenatal alcohol use, mother's marital status, maternal age, the child's sex, and birth order were also considered and found not to influence the analyses. Some studies have also considered the effects of co-exposure to other metals or toxicants. For example, Kim *et al.* (2009) demonstrated that children with higher blood manganese levels had a larger magnitude decrease in IQ for a given level of Pb; the results of this study suggest that co-exposure to other metals should be considered in studies of cognitive effects of Pb.

Shape of the dose-response curve

There is abundant discussion in the Pb literature on the shape of the dose-response curve in the lower range of exposure (i.e., at blood Pb levels <10µg/dL) for neurodevelopmental effects of Pb. This discussion is centered around a number of studies that have reported greater neurocognitive effects (principally on IQ and specific measures of cognitive function) of an incremental increase in blood Pb levels at lower concentrations compared to the effects for an incremental increase at higher blood Pb levels (Canfield *et al.* 2003a; Kordas *et al.* 2006; Lanphear *et al.* 2005; Rothenberg and Rothenberg 2005). The 2006 EPA AQCD for Lead (U.S. EPA 2006) discusses this issue extensively and reviews the evidence that the dose-response curve has a steeper slope at lower blood Pb levels. The 2005 CDC (CDC 2005) review of the epidemiological evidence for neurological effects in children also noted that there was evidence for a steeper slope in the dose-response curve at lower blood Pb levels. Evaluation of the shape of the dose-response curve is beyond the scope of the current evaluation.

Summary of support for conclusions

Animal data support a Pb-associated decrease in neurobehavioral tests of learning including fixed interval operant conditioning at blood Pb levels ≥11µg/dL (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The human data include multiple meta-analyses, prospective and cross-sectional studies that support an association between blood Pb levels <10µg/dL and lower FSIQ scores in children aged 4-13. The conclusion of *sufficient* evidence that decreases in IQ scores in children are associated with blood Pb levels <10µg/dL measured in early childhood or in concurrent blood Pb samples is based on consistent evidence for decreased IQ across multiple studies and in well accepted pooled analyses (e.g., Lanphear *et al.* 2005). Multiple studies (e.g., Baghurst *et al.* 1992; Bellinger *et al.* 1992; Min *et al.* 2009) reported that early childhood (2-4 years of age) Pb exposure is associated with IQ score in children at later ages. Clear evidence that early childhood exposure is associated with decreased IQ at later ages is complicated by the high degree of correlation in childhood blood Pb levels over time (e.g., see Dietrich *et al.* 1993a; Lanphear *et al.* 2005). The conclusion of *limited* evidence that prenatal blood Pb levels <10µg/dL are associated with decreased IQ in children is based on mixed evidence for an association with maternal or cord blood Pb. The NTP's conclusions for *sufficient* evidence that blood Pb levels <10µg/dL are associated with decreased IQ in children aged 4-13 is consistent with the conclusion from EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead.

<u>Other general and specific measures of cognitive function</u>

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with decreases in various general and specific measures of cognitive function in children from 3 months to 16 years of age (see Appendix A: Neurological Effects for full list of studies). A negative association between blood Pb level and specific cognitive abilities has been demonstrated in prospective and cross-sectional studies using a wide range of tests to assess cognitive function. Although Pb-related decreases have been reported in multiple cognitive measures including individual domains for attention, executive function, language, learning and memory, and visual-spatial processing, the lack of a clear pattern of effects has contributed to the difficulty of

discriminating focused effects because test performance covers multiple neurobehavioral processes (ATSDR 2007; U.S. EPA 2006). Although relatively few studies examined the potential effects of Pb in adults without occupational exposure, a number of studies have reported Pb-associated decreases in specific measures of cognitive function in cohorts of older adults such as the Normative Aging Study. Several of these studies reported an association between concurrent blood Pb levels <10µg/dL and decreased performance in the Mini-Mental State Examination (MMSE) and other cognitive measures. Six studies also demonstrated that bone Pb levels were associated with decreased cognitive performance. However, other studies did not find an association with concurrent blood Pb levels. There is *limited* evidence that blood Pb levels <10µg/dL are associated with decreases in specific measures of cognitive function in older adults because of the mixed results for an association with blood Pb and the stronger support for an association with bone Pb.

There are a large number of tests used to evaluate cognitive function of different aged subjects and designed to explore different cognitive domains (see Appendix A: Neurological Effects under outcomes measured for tests used in individual studies). Two of the more common tests that demonstrated effects of Pb in younger subjects include general measures of cognitive function such as the Bayley Mental Developmental Index (MDI) and the McCathy Scales of Children's Abilities including General Cognitive Index (GCI) and individual measures. Data on older children evaluated with the Wechsler Intelligence Scales for Children (WISC) were discussed earlier in the context of Pb-related decreases of IQ, which is a general measure of cognitive function; however, the WISC also includes more specific subsets such as Block Design and Digit Span and multiple studies reported decreases in WISC subsets at blood Pb levels <5µg/dL (lower than the 10µg/dL blood Pb levels generally associated with effects on FSIQ). Multiple tests are used to evaluate cognitive function in adults including the Mini-Mental State Examination (MMSE).

A negative association between blood Pb level and MDI score was demonstrated in multiple studies at blood Pb levels from <2 to 10µg/dL. Maternal blood Pb levels <10µg/dL and cord blood Pb levels <5µg/dL have been reported to be associated with decreased cognitive performance in children up to 3 years of age by the MDI. Recent studies such as (Al-Saleh *et al.* 2009; Gomaa *et al.* 2002; Jedrychowski *et al.* 2009a; Jedrychowski *et al.* 2009b; Pilsner *et al.* 2010) reported effects in children with blood Pb levels that remained consistently below 10µg/dL; however, early childhood blood Pb increased to levels above 10µg/dL for some of the cohorts examined. Two recent studies reported that concurrent blood Pb levels <10µg/dL are associated with decreased MDI scores, but there is less evidence that concurrent blood Pb is associated with MDI than for measures of prenatal Pb exposure.

The overwhelming majority of prospective studies found that prenatal exposure determined by either cord or maternal blood Pb levels <10µg/dL were associated with decreased MDI in children through 3 years of age. Significantly lower MDI in children tested at 3 to 36 months of age was significantly associated with prenatal exposure as indicated by cord blood Pb at the following levels: ≥1µg/dL in children evaluated at 24 and 36 months of age in a study of 457 children in Poland (effects significant for boys and girls combined , but likely driven by boys

Jedrychowski *et al.* 2009a; Jedrychowski *et al.* 2009b); 2.7µg/dL with MDI evaluated in 6-month-old children in Saudi Arabia (n=119) (Al-Saleh *et al.* 2009); 6.4µg/dL with MDI evaluated in 3-month-old children from the Cincinnati Lead Study (n=266) (Dietrich *et al.* 1987); 6.6µg/dL with MDI evaluated in 6, 12, 18, and 24-month-old children from Brigham and Women's Hospital (n=182-249) (Bellinger *et al.* 1986; Bellinger *et al.* 1987; Bellinger *et al.* 1984); 6.7µg/dL with MDI evaluated in 24-month-old children from Mexico City(n=197) (Gomaa *et al.* 2002; Pilsner *et al.* 2010); 9.2µg/dL evaluated at 3, 6, and 12 months of age in a study of 133 children in China (Shen *et al.* 1998). Although the majority of studies demonstrated a Pb-associated decrease in MDI, Cooney *et al.* (1989b) reported that cord and maternal blood Pb levels (mean 8-9µg/dL) in participants in the Sydney Lead study (n=215-274) were not related to change in MDI tested at 6, 12, 24, or 36 months of age.

Several prospective studies reported an association between maternal blood Pb and decreased MDI, with no relationship to cord blood or a less consistent relationship (e.g., Dietrich *et al.* 1987; Dietrich *et al.* 1990; Ernhart *et al.* 1987; Ernhart *et al.* 1988; Hu *et al.* 2006). Hu *et al.* (2006) examined maternal blood Pb during the 1st, 2nd, and 3rd trimester and found that 1st trimester blood Pb level (mean =7µg/dL) was associated with lower MDI in children at 24 months of age in a study of 146 mother-infant pairs from Mexico City; but that the association with cord blood Pb or maternal Pb during other time periods was not significant. Dietrich *et al.* (1987; 1990) reported that maternal blood Pb sampled during pregnancy (mean =8µg/dL) was associated with decreased MDI tested at 3, 6, and 24 months of age in children from the Cincinnati Lead Study and cord blood was only associated with MDI evaluated at 3 months of age. Ernhart *et al.* (1987) reported that decreased MDI evaluated at 6 months of age was associated with maternal blood Pb at delivery (mean =6.5µg/dL) in children from the Cleveland Lead Study; however, neither cord blood Pb or maternal Pb were associated with MDI evaluated at later time points (1-3 years of age). It is important to note that the blood Pb of infants from both the Cincinnati and Cleveland Lead Studies increased after birth and childhood blood Pb levels for these groups were >10µg/dL (mean =16-17µg/dL at 2 years of age), therefore, the lack of association with prenatal Pb levels may be influenced by the high childhood blood Pb levels. A follow-up study by Ernhart *et al.* (1988) did not find an association with MDI and early childhood blood Pb or concurrent blood Pb levels; however, as noted above the blood Pb levels in this cohort was >10µg/dL. In contrast, Bellinger *et al.* (1990) found that postnatal blood Pb was associated with a change in cognitive performance from age 24 months by the MDI to age 57 months by the GCI in a study with a similar age-range population.

Few studies have examined the relationship between MDI and exposure metrics other than blood Pb. Gomma *et al.* (2002) reported that maternal patellar Pb level was significantly associated with a decrease in MDI evaluated in children at 24 months of age in a study of 197 mother-infant pairs in Mexico City.

Several studies have also demonstrated that concurrent blood Pb levels <10µg/dL were associated with lower MDI scores in children from 6 to 36 months of age. For example, Solon *et al.* (2008) reported that concurrent blood Pb (mean 7.1µg/dL) was associated with decreased MDI in children from 6 to 36 months of age. Similar results (Pb-related decrease in MDI) were

reported in children evaluated at 24 months of age in Mexico City (Tellez-Rojo *et al.* 2006) with mean concurrent blood Pb level of 4.9µg/dL.

Similar to the data supporting a negative effect of blood Pb on the MDI, findings from several studies support an association with decreased performance on the General Cognitive Index (GCI) in children. In a study of 170 children from the Brigham and Women's Hospital, Bellinger *et al.* (1991) reported that GCI scores and the McCarthy subscale score for perceptual performance evaluated at 57 months of age were negative associated with blood Pb levels in the children at 24 months (mean 6.4µg/dL), but not at other ages. Similarly, Schnaas *et al.* (2000) reported that blood Pb levels from 24 to 36 months of age were associated with decreased performance on the GCI, but effects at earlier ages or later ages up to 56 months were not significant in a study of 112 children from the Mexico City Prospective study. Blood Pb was consistently below 10µg/dL in the cohorts from both the Bellinger *et al.* (1991) and Schnaas *et al.* (2000) studies. It is also important to note that some studies did not find a significant association between blood Pb and performance on the GCI; for example, maternal blood Pb (9µg/dL), cord Pb (8µg/dL), or current blood Pb were not associated with performance on the GCI in a study of 207 children from the Sydney Lead study evaluated at 48 months of age (Cooney *et al.* 1989a).

Multiples studies have reported that concurrent and early childhood blood Pb levels <10µg/dL are associated with decreases in specific indices of cognitive function in children from 4 to 16 years of age. Examples include studies demonstrating decreased performance on subsets of the Wechsler Intelligence Scales for Children (WISC). In a cross-sectional study of 384 children in Germany, Walkowiak *et al.* (1998) reported that concurrent blood Pb (mean 4.7µg/dL) in 6 year olds was associated with decreased vocabulary scores evaluated as part of the German version of the WISC. In a large cross-sectional study of children aged 6-16 from the NHANES III dataset, Lanphear *et al.* (2000) and Krieg *et al.* (2010) demonstrated that concurrent blood Pb levels <10 µg/dL (geometric mean 1.9µg/dL) were associated with decrements in the Block Design and Digit Span subsets of the WISC-R. In subgroup analysis, Min *et al.* (2009) reported that blood Pb <5µg/dL measured at 4 years of age was significantly associated with decreased performance by the Wechsler Preschool and Primary Scales of Intelligence (WPPSI-R) at 4 years of age and the perceptual reasoning scores of the WISC at age 9 in a prospective study of 278 inner-city children from Cleveland, OH. Chiodo *et al.* (2007; 2004) evaluated a range of neurocognitive endpoints in inner-city African American children aged 7-9 (n=243 and 506 respectively) with concurrent blood Pb levels (mean 5.4µg/dL); blood Pb levels were related to decreased performance in multiple tests including the Block Design and Digit Span subsets of the Wechsler Intelligence Scales for Children (WISC) and various tests of executive function, memory, and attention. When dichotomized by cut-points of 10, 7.5, 5, and 3µg/dL blood Pb; the Chiodo studies found significant effects on some tests at levels of ≥3µg/dL (e.g., Block Design).

The majority of studies of the effects of Pb on cognitive function in adults involved occupationally exposed individuals with blood Pb levels >10µg/dL, and fewer studies have been reported in adults from the general population. A number of studies in older adults reported a

Pb-associated decrease in cognitive function, with stronger evidence for an association with bone Pb than for concurrent blood Pb levels. Payton *et al.* (1998) reported that concurrent blood Pb (mean =5.5µg/dL) levels in 141 older men (mean age 67) from the Normative Aging Study were associated with decreases in specific measures of cognitive function including slower pattern comparison speed, vocabulary, word list memory, constructional praxis, and the Boston naming test among a battery of tests administered. Tibia Pb level (but not patellar Pb) was associated with decreased performance in a test of spatial ability. In a study of 736 older men (mean age 69) also from the Normative Aging study, Wright *et al.* (2003) reported that blood Pb (mean =4.5µg/dL), patellar Pb, and tibia Pb were all associated with decreased performance on the Mini-Mental State Examination (MMSE). Muldoon *et al.* (1996) evaluated cognitive performance with the MMSE and Wechsler Adult Intelligence Scale (WAIS-R) for 530 older women (mean age 71) from either a rural residence in Pennsylvania or from urban dwellers in Baltimore. Blood Pb (mean 4.8µg/dL) was associated with decreased performance on the Trailmaking (OR = 2.60(95%CI:1.04,6.49) for blood Pb >8µg/dL and OR = 2.05(95%CI:1.05,4.02) for blood Pb 4-7µg/dL relative to referents with blood Pb ≤3µg/dL) and Digit Symbol Substitution tests (OR = 3.73(95%CI:1.57,8.84) for blood Pb >8µg/dL and OR = 2.03(95%CI:1.06,3.88) for blood Pb 4-7µg/dL relative to referents with blood Pb ≤3µg/dL) in the rural population but not the urban population (Muldoon *et al.* 1996).

There are also several studies that reported an association between bone Pb and decreased performance, but did not find an association with blood Pb levels. Shih *et al.* (2006) reported a lack of an association between current blood Pb (mean 3.5µg/dL) and cognitive function in a study of 985 older adults in the Baltimore Memory Study (mean age 60). However, tibia Pb levels were significantly associated with lower scores in all 7 domains of the cognitive test battery (Shih *et al.* 2006). Weuve *et al.* (2009), reported that tibia Pb levels were associated with reduced cognitive function by the Telephone Interview for Cognitive Status in a study of 587 older women (mean age 61) from the Nurses Health Study; blood Pb and patellar Pb levels were not significantly related to the test score. Two studies did not find an association with blood Pb levels and did not collect bone Pb data. Nordberg *et al.* (2000) did not find an association between blood Pb level (mean 3.7µg/dL) and performance on the MMSE in a study of 762 older adults in Sweden with mean age of 88 years. Gao *et al.* (2008) reported that concurrent blood Pb (mean 3.9µg/dL) was not significantly related with cognitive function in as study of 188 people with mean age of 69 from rural China assessed with a test battery including the Community Screening Instrument for Dementia.

Several studies have reported a greater effect on changes in cognitive function over time, rather than a single cross-sectional examination. Weisskopf *et al.* (2004) tested cognitive function in 466 men (mean age 67) from the Normative Aging Study over several years, and reported that higher patella Pb was associated with a greater decline in performance on the MMSE over a 3.5 year period between retesting; there was no association with blood Pb (mean 4µg/dL) and the association with tibia Pb level was weaker. In an expanded study of the same population covering 1089 men, Weisskopf *et al.* (2007) reported similar results, stating that there was little association between blood or bone Pb levels and cognitive test scores on a cross-sectional basis; however, patellar and tibia Pb levels were associated with decline in

performance on a range of cognitive function, particularly visuospatial and visuomotor subscales. Bandeen-Roche (2009) reported that tibia Pb was associated with decreased hand-eye coordination over time in a study of 964 older adults from the Baltimore Memory Study (age 59 at baseline), but not other measures of cognitive function in a battery of 20 standardized tests.

Fewer studies have examined cognitive performance in younger adults with low blood Pb levels. In a series of studies from Krieg *et al.* in 4937 adults aged 20-59 from the NHANES III dataset, blood Pb levels <10µg/dL in adults were not associated with performance on neurobehavioral tests. Krieg *et al.* (2009; Krieg *et al.* 2010; Krieg *et al.* 2009; 2005) did not find a significant relationship between blood Pb (mean 3.3µg/dL) and neurobehavioral tests for simple reaction time, symbol-digit substitution, and serial digit learning. In a portion of the study that included both children and adults from the same NHANES dataset, Krieg *et al.* (2010) demonstrated that *VDR* genotype did effect the relationship between blood Pb and performance on the WISC-R Digit Span and WRAT math scores; however, they found no clear pattern in terms of the effect of *VDR* genotype on the relationship between blood Pb and cognitive function.

The majority of recent studies of the relationship between blood Pb and specific measures of cognitive function considered a range of potential confounders such as age, sex, education, and race/ethnicity. Some studies have also examined potential physical, genetic, and psychological confounders. In a study of 47 health adults aged 55-67 in Rochester, NY, van Wijngaarden *et al.* (2009) found that higher tibia and calcaneus Pb levels were significantly correlated with measures of memory impairment; however, the relationship with bone Pb was not significant after adjusting for hypertension. Several studies of cognitive function in children and adults have also investigated the potential modifying effect of gene polymorphisms (e.g., *ALAD*, *HFE*, *APOE*, and *VDR* genotypes) and other factors (Glass *et al.* 2009; Krieg *et al.* 2010; Rajan *et al.* 2008; Weuve *et al.* 2006). In particular, Wang *et al.* (2007) demonstrated a significant effect of HFE polymorphism on the rate of decline in MMSE score in 358 participants from the Normative Aging Study. Krieg *et al.* (2010) and Rajan *et al.* (2008) reported that there was no clear pattern of ALAD or VDR genes modifying the relationship of Pb and cognitive function. Glass *et al.* (2009) found that tibia Pb was associated with impaired executive function and there was a significant interaction with neighborhood psychosocial hazards in a study of 1001 older adults (mean age 59) from the Baltimore Memory Study. Surkan *et al.* (2008) reported that maternal self-esteem attenuated the Pb-associated decrease in MDI score in a study of 309 children aged 2 from Mexico City. Peters *et al.* (2010) reported that blood Pb (mean 5µg/dL) was significantly associated with decreased cognition by the MMSE, but that bone Pb and stress were modifiers of the association with Pb in a study of 811 older men (mean age 68) in Normative Aging Study.

Summary of support for conclusions

Animal data support a Pb-associated decrease in neurobehavioral tests of learning including fixed interval operant conditioning at blood Pb levels ≥11µg/dL and mixed evidence for effects on memory (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The human

data include multiple prospective and cross-sectional studies supporting a Pb-associated decrease in specific measures of cognitive function in children at blood Pb levels from 1 to 10µg/dL. The conclusion of *sufficient* evidence for decreases in specific measures of cognitive function in children aged 3 months to 16 years of age with blood Pb levels <5µg/dL measured in concurrent blood or in early childhood is based on the consistency of effects on multiple measures of cognitive function in multiple studies. Multiple studies (e.g., Min *et al.* 2009) reported that early childhood Pb exposure is associated with decreases in cognitive function observed at later ages. However, as discussed for IQ, clear evidence that early childhood or prenatal Pb exposure is associated with decreases in specific indices of cognitive function at later ages is complicated by the high degree of correlation in childhood blood Pb levels over time (e.g., see Dietrich *et al.* 1993a; Lanphear *et al.* 2005). Multiple studies (e.g., Al-Saleh *et al.* 2009; Bellinger *et al.* 1984; Jedrychowski *et al.* 2009a) also reported that cord blood Pb levels are associated with decreases in cognitive function observed at later ages by cognitive measures such as the MDI. The conclusion of *limited* evidence that prenatal blood Pb levels <5µg/dL are associated decreases in specific measures of cognitive function in children is based on strong support for an association between cord blood MDI and the mixed evidence for maternal blood Pb and MDI or other measures of cognitive function in children. The conclusion of *limited* evidence that blood Pb levels <10µg/dL are associated with decreases in specific measures of cognitive function in older adults is based on the mixed evidence that concurrent blood Pb levels <10µg/dL are associated with reduced cognitive function and the consistent support that bone Pb is associated with decreases in specific measures of cognitive function or with change in these measures through time in older adults. The EPA's 2006 AQCD for Lead suggests that cumulative exposure to Pb may be critical in contributing to neurocognitive deficits in adults because of the significant associations with bone Pb; EPA also highlights the mixed evidence for an association with blood Pb. As with other studies of health effects of Pb in adults, prospective studies in a population for which the data demonstrated that blood Pb levels remained consistently below 10µg/dL from birth until measurement of the outcome of interest would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL. The NTP's conclusion that there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with decreases in general and specific measures of cognitive function in children aged 3 months to 16 years extends the conclusion from EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead which were limited to blood Pb levels <10µg/dL; however, the EPA's 2011 draft (U.S. EPA 2011) currently supports a lower blood Pb level.

4.3.2 Behavior

Attention Deficit Hyperactivity Disorder (ADHD)

There is *sufficient* evidence that blood Pb levels <5 µg/dL are associated with diagnosis of ADHD in children aged 3 to 18 (see Appendix A: Neurological Effects for full list of studies). Most of the studies that demonstrated a significant association between Pb and ADHD are studies with behavioral testing and concurrent blood Pb levels. Increased diagnosis of ADHD and ADHD-related behaviors such as inattention and hyperactivity are consistently reported in studies with mean blood Pb levels <5µg/dL and in several studies at blood Pb levels <2µg/dL. The diagnostic

criteria for ADHD include multiple categories of behavioral deficits in addition to inattention, hyperactivity, and the overall diagnosis of ADHD (Aguiar *et al.* 2010). Additional review of individual behavioral domains such as response inhibition, flexibility, and planning aspects was recently published by Eubig *et al.* (2010); however, data on those individual domains will not be covered here because there are sufficient studies of overall ADHD diagnosis, inattention, and hyperactivity to develop conclusions. There is *limited* evidence that prenatal blood Pb levels ≤10µg/dL are associated with behavioral features of ADHD in childhood. Several prospective studies reported that Pb exposure determined from maternal blood Pb during pregnancy, cord blood, early childhood blood Pb (up to 30 months), or from tooth dentin levels (6-8 years of age) is associated with inattention or hyperactivity as children or young adults[2] (aged 7-20); however, early childhood blood Pb levels in some of these individuals are >10µg/dL; therefore, the effect may not be strictly associated with blood Pb levels below 10µg/dL.

The clear majority of recent cross-sectional studies (more than 10 publications since 2000), demonstrated an association between current blood Pb at mean levels from 1 to 11µg/dL and diagnosis of ADHD or other indicators of decreased attention or increased hypersensitivity in children from 3 to 18 years of age. Multiple studies reported an association with blood Pb of 5µg/dL and below. In a case-control study of 1260 children (630 ADHD cases) in China ages 4-12, Wang *et al.* (2008) reported a significant association between concurrent blood Pb levels >5µg/dL and ADHD from a structured diagnostic interview. An increase in ADHD–related behaviors, particularly inattention, was observed on the teachers' and parents' rating using the Connors scale and German-KITAP battery in a study of 83 children in Romania ages 8-12 with concurrent mean blood Pb levels 3-5µg/dL (Nicolescu *et al.* 2010). Chiodo *et al.* (2007; 2004) evaluated a range of neurodevelopmental endpoints in inner-city African American children aged 7-9 (n=243 and 506 respectively) with concurrent blood Pb levels (mean 5.4µg/dL); blood Pb levels were related to higher ADHD and inattention scores on the Barkley-DuPaul Scale, greater hyperactivity on the PROBS-14 scale, and poor attention on the Teacher Report Form. When dichotomized by cut-points of 10, 7.5, 5, and 3µg/dL blood Pb the Chiodo studies supported significant effects on inattention at blood Pb levels of ≥3µg/dL.

Two publications that evaluated ADHD in children from the NHANES (1999-2002) dataset (Braun *et al.* 2006; Froehlich *et al.* 2009) reported a positive association between concurrent blood Pb levels of 1-2µg/dL and ADHD. Braun *et al.* (2006) reported a significant increase in the odds ratio OR=4.1(95% CI:1.2,14.0; p=0.001) for ADHD among children ages 4-15 with blood Pb levels >2µg/dL compared to referents with blood Pb 0.8µg/dL (n= 4704 participants; ADHD based on parental reported previous diagnosis or use of stimulant medication). In children aged 8 to 15 from the same NHANES dataset (n=2588), Froehlich *et al.* (2009) found that children with concurrent blood Pb >1.3µg/dL (the 3rd tertile of Pb exposure) had a significantly greater odds ratio OR=2.3(95% CI:1.5,3.8; p=0.001) and children with blood Pb ≥0.9-1.3µg/dL had an OR=1.7(95% CI:0.97,2.9; p=0.06) for ADHD by the Diagnostic Interview Schedule for Children compared with referents (the first tertile of Pb exposure) with 0.8µg/dL blood Pb.

[2] Note: children are defined as <18 years of age in this document; therefore 18-20 year olds in the study are considered adults

Several other cross-sectional studies support an association between concurrent blood Pb levels of ≤2μg/dL and ADHD and symptoms of inattention and or hyperactivity. In a cross-sectional study of 639 children aged 8-11 in Korea, Cho *et al.* (2010) reported a significant association between blood Pb levels (mean1.9μg/dL) and ADHD rating in inattention, hyperactivity, and total score using the teacher evaluation of the ADHD Rating Scales. Concurrent blood Pb level (mean 1.8μg/dL) was positively associated with ADHD by the Conner's scale for ADHD in a study of 1778 children aged 7 in the South Korean Children's Health and Environment Research Study (Ha *et al.* 2009). In a pair of studies of 236 children, Nigg *et al.* (2008; 2010) reported that concurrent blood Pb levels were significantly higher in ADHD-combined type children aged 6-17 than controls and that blood Pb levels (mean 1μg/dL) were significantly correlated with ADHD diagnosis, hyperactivity, oppositional behaviors, ADHD index, and attention problems evaluated with the Conners' Rating Scale. Nigg *et al.* (2008; 2010) found that measures of hyperactivity-impulsivity were more consistently associated with blood Pb measurements than were inattention symptoms.

However, the consistent association with hyperactivity over inattention observed by Nigg *et al.* (2008; 2010) is not universal, and several other studies have found an association with blood Pb and ADHD or measures of inattention. Kim *et al.* (2010) found increased inattention and hyperactivity symptoms on the teacher-completed ADHD rating scale in children with blood Pb ≥2.2μg/dL compared to referents with lower blood Pb levels in a study of 275 South Korean children aged 8-10. Roy *et al.* (2009) reported an increase in the ADHD index (β=0.17; p=0.05) and DSM-IV inattentive (β=0.24; p=0.01), but not DSM-IV hyperactive (β=0.17; p=0.13) by the Connors' ADHD /Diagnostic and Statistical Manual for Mental Disorders (4^{th} edition) scales in 3-7-year-old children in India with concurrent mean blood Pb of 11μg/dL; increased anxiety and social problems were also noted. Inattention and hyperactivity in 11-year-olds evaluated by the Parents' and Teachers' Hyperactivity and Inattention scales were also correlated with blood Pb (mean 11 μg/dL) in a cross-sectional study of 579 children from the Dunedin Multidisciplinary Health and Development Study in New Zealand (Silva *et al.* 1988). Canfield *et al.* (2003b) reported that blood Pb at 4 years of age was associated with decreased focused attention by the Shape School at 4-5 years of age in 172 children in Rochester, NY.

The relationship between concurrent blood Pb levels and ADHD is supported by multiple studies, but there are also data supporting a role for early-life prenatal or early-childhood Pb exposure and behaviors associated with ADHD in older children. In a prospective study with Pb exposure measures spanning prenatal and early childhood to 6 years of age, Ris *et al.* (2004) reported that attention in 15-17-year-old children by the Continuous Performance Test was negatively associated with maternal blood Pb during the 1^{st} or 2^{nd} trimester (mean 8.9μg/dL), average childhood blood Pb <5 years of age, and blood Pb at 6.5 years of age in a study of 195 children from the Cincinnati Pb Study); however, blood Pb levels during early childhood were above 10μg/dL in this cohort (Dietrich *et al.* 1993a; Wright *et al.* 2008) so it is not clear that this is strictly associated with blood Pb levels <10μg/dL. Chandramouli *et al.* (2009) found that attention in 7-8-year olds in the United Kingdom assessed with the Test of Everyday Attention for Children was not significantly related to blood Pb (mean 4.2μg/dL) at 30 months of age (n=582), but that there was a greater odds ratio for teacher-rated hyperactivity

OR=2.82(95%CI:1.08, 7.35) in children with blood Pb >10µg/dL. Cord blood Pb (mean 6.8µg/dL) and tooth dentin Pb were associated with inflexible behavior in 8-year olds in the Task domain of the Boston Teachers Questionnaire, but there was no relationship with hyperactivity in the study of 1923 children in Boston (Leviton *et al.* 1993). Fergusson (1993) reported a significant association between tooth dentin Pb of shed teeth (ages 6-8) and measures of inattention and restlessness at 12-13 years of age by the Rutter and Conners parental and teacher questionnaires in a study of 1265 children from the Christchurch Health and Development Study cohort. In a similar study of 79 young adults[3] aged 19-20, Bellinger *et al.* (1994a) reported that both dentin Pb levels in shed teeth (6-8 years of age) and tibia Pb levels were significantly associated with specific measures of attention.

Summary of support for conclusions

Animal data support a Pb-associated reduced performance on neurobehavioral tasks including increased distractibility with effects observed down to approximately 10µg/dL (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The human data supporting a Pb-associated increase in ADHD diagnosis and behavioral features of ADHD such as inattention and hyperactivity include multiple cross-sectional studies of children from 3 to 18 years of age with blood Pb levels of 1-11µg/dL. The conclusion of *sufficient* evidence for a positive association with ADHD in children at blood Pb levels <5µg/dL is based on the consistency of effects in these studies and supports effects down to and below 2µg/dL blood Pb. These conclusions are based largely on the parent reporting a physician's diagnosis or that the child is taking ADHD medication; therefore, they lack the additional strength that would be provided by studies that incorporate diagnostic evaluations to identify ADHD using DSM-IV-TR criteria. Recent studies found effects on ADHD after controlling for a large number of confounders such as socioeconomic variables, sex, race/ethnicity, age of blood Pb measurement, parental education, and tobacco exposure. Several studies reported that blood Pb levels were significantly associated with ADHD even after controlling for potential mediating effects of child IQ (e.g., Nigg *et al.* 2008; Nigg *et al.* 2010). Although several studies reported an association between concurrent blood Pb and ADHD in children up to ages 15 or 17 (Braun *et al.* 2006; Froehlich *et al.* 2009; Nigg *et al.* 2008; Nigg *et al.* 2010), or between bone Pb measured as children and attention evaluated as young adults age 19-20 (Bellinger *et al.* 1994a), no studies were located that examined the relationship between blood Pb levels in adults and ADHD. There is *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL and effects on ADHD in adults. The conclusion of *limited* evidence for ADHD in children with prenatal Pb exposure at blood Pb levels <10µg/dL is based on the evidence for an association with prenatal blood Pb <10µg/dL that may include childhood exposure >10µg/dL with support from the concurrent blood Pb data and a number of bone Pb studies reporting an association with ADHD-related behaviors such as inattention or hyperactivity. Existing data supports the importance of current Pb exposure for ADHD, but does not allow a clear distinction to be made as to the role of early life exposure. The NTP's conclusions for *sufficient* evidence that ADHD in children aged 3-18 is associated with Pb levels <5µg/dL, is stronger than the limited relationship

[3] Note: children are defined as <18 years of age in this document; therefore 19 and 20 year olds in the study are considered adults

with behavioral features of ADHD outlined in EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead; however, given the growth of the database in recent years the EPA's 2011 draft (U.S. EPA 2011) currently supports an association with ADHD.

Problem Behaviors

The discussion of problem behaviors below is divided into two sections focused on: 1) delinquent, criminal, or antisocial behavior; and 2) psychiatric outcomes. The majority of the studies of Pb effects on problem behaviors is focused on studies of conduct problems or criminal behavior. Recent studies of mood disorders or psychiatric outcomes such as anxiety and depression have also reported an association with blood Pb levels.

Delinquent, Criminal or Antisocial Behaviors

There is *sufficient* evidence that blood Pb levels <5 μg/dL are associated with antisocial behavioral problems or actual criminal behavior in children from 6 to 15 years of age (see Appendix A: Neurological Effects for full list of studies). Recent studies, including studies with large sample sizes such as the NHANES datasets, have reported effects down to blood Pb levels <1μg/dL and a number of cross-sectional studies demonstrated an association between concurrent blood Pb at and below 10μg/dL and antisocial problem behaviors. Multiple studies reported that bone Pb and tooth dentin Pb are related to antisocial behavior problems. There is *limited* evidence that prenatal blood Pb levels <10μg/dL are associated behavioral problems in childhood. A number of prospective studies have reported a significant association between prenatal blood Pb <10μg/dL and delinquent behavior or criminal arrests as children; however, childhood blood Pb levels in some of these individuals are >10μg/dL; therefore, the effect may not be strictly associated with blood Pb levels below 10μg/dL. Several studies have also reported that cord blood Pb was not associated with antisocial problem behaviors in children. Although several studies have reported an association between prenatal Pb levels <10μg/dL and criminal arrests in young adults[4] up to age 24, no studies of antisocial behavioral problems were located with Pb levels in adults. There is *inadequate* evidence to evaluate the potential association between blood Pb <10μg/dL and effects on behavior problems in adults.

Braun *et al.* (2008) reported a significant increase in the odds ratio OR=7.24(95% CI:1.1,49.5) for conduct disorder among children ages 8-15 with blood Pb levels ≥0.8μg/dL compared to referents with blood Pb ≤0.7μg/dL (conduct disorder measured using criteria described in the 4th edition of the Diagnostic and Statistical Manual for Mental Disorders; n= 3081 from the NHANES 2001-2004 dataset). This study extended the findings from earlier cross-sectional studies that found associations between current blood Pb at and above 10μg/dL and behavioral problems or criminal behavior in children from 6 to 13 years of age (i.e., mean blood Pb 10-15μg/dL in Burns *et al.* 1999; Silva *et al.* 1988; Thomson *et al.* 1989; Yule *et al.* 1984). For example, in a cross-sectional study of 11-year-old children (n=579) in New Zealand, blood Pb (mean 11μg/dL) was significantly correlated with behavioral problems on the Parent's and Teachers' Rutter Behavioral Scales (Silva *et al.* 1988). Burns *et al.* (1999) reported that

[4] Note: children are defined as <18 years of age in this document; therefore 19-24 year olds in these studies are considered adults

increased behavioral problem scores by the Achenbach Child Behavior Checklist were associated with lifetime average blood Pb (geometric mean 14 µg/dL) in a study of 322 children age 11-13 from the Port Pirie birth cohort.

Several prospective studies support an effect of Pb exposure on antisocial problem behaviors using Pb exposure determined by blood Pb or bone Pb (Bellinger *et al.* 1994b; Chen *et al.* 2007; Dietrich *et al.* 2001; Wasserman *et al.* 2001). Results of some studies support a stronger effect of bone Pb or concurrent blood Pb than prenatal or early childhood blood Pb with behavioral outcomes. For example, Bellinger *et al.* (1994b) reported that problem behaviors in 8-year-old children in Boston (n=1782 judged by the Teacher Report Form of the Child Behavior Profile) were significantly associated with tooth dentin Pb levels; however, there was no association with cord blood Pb levels (mean 7µg/dL). Behavioral measures in a study of 7-year-old children (n=780) demonstrated an association with concurrent blood Pb (mean 8µg/dL) for both indirect effects on behavior symptoms mediated through IQ and direct effects on behavior scores or indices evaluated with the Behavioral Assessment System for Children (BASC) scores for school problems and behavioral symptoms index (Chen *et al.* 2007); however, there was no association with earlier blood Pb levels that were significantly above 10µg/dL at age 2 years (mean 26µg/dL) or 5 years (mean 12µg/dL).

In contrast, other prospective studies support an association with prenatal or early childhood exposure with problem behaviors at a later age. Dietrich *et al.* (2001) found significant associations between maternal blood Pb (mean 8.9µg/dL) and childhood blood Pb measures ≤7 years of age with Self-Report of Delinquent Behavior in children from the Cincinnati Lead Study evaluated at ages 15-17 (concurrent mean 3µg/dL); however, as in Chen *et al.* (2007) blood Pb levels during early childhood were above 10µg/dL so it is not clear that this is strictly associated with blood Pb levels <10µg/dL. Similar results were reported for cord blood and concurrent blood Pb and behavior problems in 3-year olds from the Yugoslavia Prospective Study; however, blood Pb levels (mean 5.5 in Pristina and 22µg/dL in Mitrovica) in many children in this study were also above 10µg/dL (Wasserman *et al.* 1998). Chandramouli *et al.* (2009) found that the odds ratio for antisocial behaviors OR=2.90(95%CI:1.05,8.03) in 7-8 year olds (n=582) in the United Kingdom assessed by the Anti-social Behavior Interview was significantly elevated with blood Pb >10µg/dL at 30 months of age. Two studies have also reported an association with prenatal or childhood blood Pb levels and antisocial behavior problems as young adults[5]. The rate ratios for total criminal arrests in 19-24 year olds for a 5µg/dL increase in blood Pb were significantly positively associated with maternal blood Pb during the 1st or early 2nd trimester (mean 8.3µg/dL) RR=1.4(95%CI:1.07,1.85) as well as blood Pb at 6 years of age (mean 8.3µg/dL) RR=1.27(95%CI:1.03,1.57) in a study of 250 young adults from the Cincinnati Lead Study (Wright *et al.* 2008). Hornung *et al.* (2009) reported that blood Pb at 6 years of age was more strongly related to adult criminal arrests (age not reported) than blood Pb at 2 years of age in a similar analysis reported for the combined cohort from the Cincinnati and Rochester Lead Studies with a peak blood Pb mean of 13.6µg/dL and concurrent blood Pb at 6 years of age

[5] Note: children are defined as <18 years of age in this document; therefore 19-24 year olds in the following studies are considered adults

of 6µg/dL; there was a strong correlation between the blood Pb ratio for 6 years to 2 years and criminal arrests (β=1.21; p<0.001).

Several studies support an association between higher bone Pb, tooth dentin Pb, or hair Pb and antisocial problem behaviors, but did not provide blood Pb measurements for comparison. Bone Pb levels were associated with antisocial behavior including delinquency and aggression in a study of 212 boys tested by the Child Behavior Checklist at ages 7 and 11 (Needleman *et al.* 1996). Needleman *et al.* (2002) found that tibia bone Pb in 12-18-year olds was associated with an increased odds ratio for adjudicated delinquent behavior OR=3.7(95%CI:1.3,10.5) in a case-control study of 194 case and 146 non-delinquent youths from the same high schools in Pennsylvania. Fergusson *et al.* (2008) reported a significant association between tooth dentin Pb of shed teeth (ages 6-8) and officially reported crime in a study of 1265 young adults from the Christchurch Health and Development Study cohort at age 21.

A meta-analysis of 19 studies of Pb exposure and conduct problems in children 3 to 18 years of age reported an overall r=0.19 (p<0.001) or medium effect size across the 19 studies evaluated consisting of 8,561 total children (Marcus *et al.* 2010). Although conduct problems were more common in boys than girls, the percentage of boys in the study did not appear to attenuate the relationship with Pb, nor did adjustment for other confounders such as age, socioeconomic status, parental IQ, or home environment. Marcus *et al.* (2010) note that effects were similar whether Pb exposure was measured by blood Pb, or bone Pb measured in tooth dentin or with K-x-ray analysis; however, a larger effect of hair Pb was found in the three studies from the same laboratory (Marlowe and Bliss 1993; Marlowe and Errera 1982; Marlowe *et al.* 1985) that used hair as the exposure metric. Marcus *et al.* (2010) cannot explain why the hair Pb data displayed a stronger relationship and noted that multiple studies have determined that hair Pb is less accurate than blood Pb measurements for determining Pb exposure (e.g., ATSDR 2001 and see discussion in the Section 3.1 Biomarkers of Exposure). This suggests that the Marlowe *et al.* studies contain a bias or other population factor that may explain the stronger relationship in these studies.

Summary of support for conclusions

Animal data support a Pb-associated neurobehavioral deficits, including reduced ability to inhibit inappropriate responding, at blood Pb levels close to levels reported in human studies (i.e., approximately 10µg/dL) (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The human data from multiple prospective and cross-sectional studies support a Pb-associated increase in antisocial problem behaviors or criminal behavior in children from 6 to 15 years of age with concurrent blood Pb levels of <1-15µg/dL. The conclusion of *sufficient* evidence for a positive association with behavior problems in children at blood Pb levels <5µg/dL is based on the consistency of effects in these studies and the Braun *et al.* (2008) study from the NHANES 2001-2004 dataset that reported conduct disorder at blood Pb levels ≥0.8µg/dL blood Pb. Most of the recent studies in the database include a large number of confounders such as socioeconomic variables, sex, race/ethnicity, age of blood Pb measurement, parental education, and tobacco exposure. Several studies reported that blood Pb levels were significantly associated with antisocial behavioral problems even after

controlling for child IQ through model adjustments or by path analysis (Burns *et al.* 1999; Chen *et al.* 2007; Silva *et al.* 1988). Although the Wright *et al.* (2008) study reported that criminal arrests in young adults aged 19-24 were associated with prenatal and childhood blood Pb levels, and the Fergusson *et al.* (2008) study found an association between reported crimes in 21-year olds and dentin Pb of shed teeth at ages 6-8, no studies of antisocial behavioral problems were located with Pb levels in adults. There is *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL and effects on behavior problems in adults. The conclusion of *limited* evidence for a positive association between prenatal Pb exposure to blood Pb <10µg/dL and behavioral problems in children is based on the mixed results of studies with prenatal exposure data. The NTP's conclusions of *sufficient* evidence that antisocial behavior problems in children are associated with Pb levels <5µg/dL, is stronger than the discussion of Pb effects on mood and behavior that may extend into increased risk for delinquent behavior described in EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead; however, given the growth of the database in recent years the EPA's 2011 draft (U.S. EPA 2011) currently supports an association with problem behavior.

Psychiatric Outcomes including Anxiety and Depression

There is *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL and anxiety or depression-related psychiatric outcomes in children (see Appendix A: Neurological Effects for full list of studies). There is *limited* evidence that blood Pb <10µg/dL in adults is associated with psychiatric symptoms including anxiety and depression. Although there are relatively few studies of children or adults with low blood Pb levels that address these mood symptoms, the available data in adults are from several large cross-sectional studies (n=526 to 1987) and support an effect of concurrent blood Pb or bone Pb on these psychiatric outcomes.

Most studies of behavior in children that have identified an association with Pb exposure have been in the area of ADHD-related behaviors or in conduct problems. However, several studies have reported an association between blood Pb at and above 10µg/dL and anxiety or depression-related behaviors in children. Wasserman *et al.* (1998) reported that cord blood Pb (mean 5.5 in Pristina and 22µg/dL in Mitrovica) and concurrent blood Pb levels in 3-year olds were associated with somatic problems, anxious-depressed and withdrawn behavior by the Child Behavior Checklist in a study of 293 children from the Yugoslavia Prospective Study; however, blood Pb levels in many children in this study were above 10µg/dL so it effects may not be associated with blood Pb levels <10µg/dL. Roy *et al.* (2009) reported increased anxiety and social problems by teacher evaluation using the Connors' Teacher Rating Scales-39 in 3-7-year-old children in India with concurrent mean blood Pb of 11µg/dL. In a small study of 42 children aged 3-5, blood Pb (mean 2µg/dL) were inversely associated with teachers rating of social confidence and sociability in girls, but Pb was not related to measures of anxiety or aggression in boys or girls (Hubbs-Tait *et al.* 2007).

Several studies have demonstrated an association between concurrent blood Pb <10µg/dL in adults and psychiatric symptoms including anxiety, depression, and panic disorder. These studies do not include cohorts in which it has been demonstrated that blood Pb levels were

consistently below 10µg/dL from birth to behavioral assessment. Bouchard *et al.* (2009) reported a significant increase in the odds ratio for diagnoses of major depression disorder OR=2.32(95%CI:1.13,4.75) and panic disorder OR=4.94(95%CI:1.32,18.48) at concurrent blood Pb levels of ≥2.11µg/dL in a study of 1987 adults aged 20-39 from the NHANES 1999-2004 dataset; a diagnosis of generalized anxiety disorder was not associated with blood Pb levels in this study. In a study of 526 men in the Normative Aging Study (mean age 67), blood Pb (mean 6.3µg/dL), tibia Pb (mean 22µg/g), and patella Pb (mean 32µg/g) were significantly associated with combined measure of mood including elevated anxiety, depression, and phobic anxiety (Rhodes *et al.* 2003). In a further study of 744 men from the Normative Aging Study, an interquartile increase in tibia bone Pb (14µg/g) or patella Pb (20µg/g) were associated with increased risk of psychiatric symptoms of somatization and global severity index (Rajan *et al.* 2007).

Summary of support for conclusions

There are some examples of Pb-associated increases in depression-related outcomes in rats and mice at blood Pb levels down to 17µg/dL (see ATSDR 2007; e.g., Dyatlov and Lawrence 2002; U.S. EPA 2006 for recent reviews of the animal data). The dataset of human studies to evaluate the association with psychiatric outcomes is relatively small for both children and adults; however, several studies in adults support an effect of concurrent blood Pb <10µg/dL or bone Pb. The conclusion of *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL in children and anxiety or depression-related psychiatric outcomes is based on the lack of studies at blood Pb levels <10µg/dL. The conclusion of *limited* evidence that concurrent adult blood Pb levels <10µg/dL are associated with psychiatric symptoms including anxiety and depression is due to the small number of studies supporting an effect (one at blood Pb <10µg/dL and one at blood Pb<5µg/dL) and that two of the three studies are from a single cohort, the Normative Aging Study. As with other studies of health effects of Pb in adults, prospective studies in a population for which the data demonstrated that blood Pb levels remained consistently below 10µg/dL from birth until assessment of anxiety, depression, or other psychiatric outcomes would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) discuss evidence that effects of Pb may extend into increased risk for antisocial and delinquent behavior. The 2006 EPA AQCD for Lead (U.S. EPA 2006) does not have specific conclusions on the potential association between Pb exposure and anxiety. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) highlights the evidence for neurobehavioral effects in older adults at blood Pb levels >4µg/dL.

4.3.3 Neurodegeneration

Amyotrophic Lateral Sclerosis (ALS)

There is *limited* evidence that blood Pb levels <10µg/dL are associated with increased risk for amyotrophic lateral sclerosis (ALS). A number of case-control studies have reported a significant association between blood Pb and ALS diagnosis with most of the studies coming from two populations (see Appendix A: Neurological Effects for full list of studies). The data

present limited evidence because of issues with potential reverse causality due to an association between ALS and bone turnover that might lead to higher Pb levels among ALS patients and bias due to a reported increased survival time in ALS patients that might also lead to higher Pb levels.

In a small case-control study of 19 ALS patients and 39 controls (mean age 64-66), mean blood Pb was not significantly different (p=0.38) between ALS patients (12.7µg/dL) and controls (10.8µg/dL) (Vinceti *et al.* 1997). The relative risk of ALS was significantly associated with blood Pb as a continuous measure OR=1.9(95%CI:1.4,2.6) or categorical variable of blood Pb 3-4µg/dL OR=14.3(95%CI:3,69), but the association with bone Pb was not significant in a case-control study of 109 ALS patients and 256 matched controls in New England (Kamel *et al.* 2002). The study reported that cases reported greater odds of having a job with Pb exposure OR=1.9(95%CI:1.1,3.3) and odds ratio for ALS was significantly associated with lifetime days of Pb exposure greater than 2000 hours OR=2.3(1.1, 4.9). The significant association with lifetime exposure is interesting because bone Pb was not significantly associated with ALS in the study and bone Pb is considered a better measure of cumulative exposure. The issue of reverse causality (the possibility that increased blood Pb is related to greater bone Pb mobilization from the reduced physical activity in ALS patients) was examined in a separate case-control study of 184 ALS cases and 194 controls among US veterans (Fang *et al.* 2010). Fang *et al.* (2010) reported that blood Pb among the ALS patients (mean 2.4µg/dL) was significantly higher than controls (1.8µg/dL). The odds ratio for ALS was significantly associated with blood Pb with OR=2.6(95%CI:1.9,3.7) for a doubling of blood Pb. The study examined the potential influence of markers of bone turnover and genetic factors and reported a significant interaction between blood Pb – ALS and plasma biomarkers for bone turnover (procollagen type-1 amino-terminal peptide (PINP)) and resorption (C-terminal telopeptides of type 1 collagen (CTX)), but not the K59N *ALAD* gene polymorphisms (Fang *et al.* 2010). However, adjusting for biomarkers of bone turnover did alter the association between blood Pb and ALS to a large degree. The authors state that reverse causality is unlikely because the Pb-ALS association persisted after adjusting for biomarkers of Pb mobilization from bone, but that reverse causality cannot be entirely ruled out because of the cross-sectional nature of the data.

In further study of the population in New England, Kamel *et al.* (Kamel *et al.* 2005; 2003) reported that the *ALAD* gene was associated with altered bone Pb levels, but not with blood Pb, and the effect on ALS was not significant. In a second, follow-up study, Kamel *et al.* (2008) demonstrated that tibia bone Pb was significantly associated with greater survival time between diagnosis and death HR=0.3 (95% CI:0.1,0.7), while patella Pb HR=0.5 (95% CI:0.2,1.0) and blood Pb HR=0.9 (95% CI:0.8,1.0) were associated with greater survival time with borderline significance. The association of bone and blood Pb with greater survival time in ALS patients introduces the possibility of bias because the case groups in these case-controls studies may be individuals with longer survival time and higher Pb levels may related to a longer period of exposure or more time for Pb to be released from bone stores.

Summary of support for conclusions

Several recent animal studies have demonstrated a similar finding to the human data supporting a relationship between Pb and ALS. For example, Barbeito *et al.* (2010) reported that blood Pb levels of 27µg/dL were associated with increased survival time in a mouse model of severe ALS; analogous to the longer survival time in ALS patients with higher blood Pb levels observed by Kamel *et al.* (2008). The NTP concluded that there is *limited* evidence that blood Pb levels <10µg/dL are associated with diagnosis of ALS because the case-control studies that reported an association with blood Pb have potential issues with reverse causality and bias due to a reported increased survival time in ALS patients, both of which might also lead to higher Pb levels in ALS patients. The data from Fang *et al.* (2010) addressed some of the reverse causality issues by controlling for factors associated with bone turnover. As with other studies of health effects of Pb in adults, studies that demonstrated an association between ALS and blood Pb in a population for which the data demonstrated that blood Pb levels remained consistently below 10µg/dL from birth until diagnosis of ALS would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL. The NTP's conclusions for *limited* evidence that blood Pb levels <10µg/dL are associated with diagnosis of ALS is consistent with the four studies highlighted in EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead.

Alzheimer's disease

There is *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL and Alzheimer's disease as no studies examining Alzheimer's disease were located in groups with blood Pb levels <10µg/dL. Although the ATSDR's 2007 Toxicological Profile for Lead and EPA's 2006 AQCD for Lead have similar conclusions on the lack of epidemiological evidence for an association between Pb and Alzheimer's disease, the EPA's 2006 AQCD for Lead highlights the evidence from studies of laboratory animals that early life exposure to Pb is associated with Alzheimer's like pathologies at later ages. In particular, developmental exposure of rats (Basha *et al.* 2005) and monkeys (Zawia and Basha 2005) to Pb was associated with overexpression of the Alzheimer's-associated amyloid precursor protein; however, Pb exposure as adults did not result in increased amyloid deposits in the brain.

Essential tremor

There is *limited* evidence that blood Pb levels <5µg/dL in adults are associated with increased risk for diagnosis of essential tremor (ET). A number of case-control studies have reported a significant association between blood Pb and essential tremor diagnosis with the majority of the studies coming from New York and similar results reported for a population in Turkey (see Appendix A: Neurological Effects for full list of studies). The data present consistent evidence for an association between blood Pb and essential tremor, but reflect a total sample size of only approximately 300 cases of essential tremor.

Louis *et al.* (2003) reported that blood Pb was significantly higher in ET patients than controls (3.3µg/dL compared to 2.7µg/dL in controls) in a case-control study of 100 ET cases and 143 matched controls from New York (mean ages 66-71). Blood Pb was significantly associated with an increased odds ratio for essential tremor diagnosis OR=1.21(95% CI:1, 1.39). Louis and

colleagues (2005; 2011) also examined the interaction between blood Pb and other modifying factors such as *ALAD* genetic polymorphisms and harmane (a β-carboline alkaloid associated with ET) exposure in this New York cohort. The odds of essential tremor were significantly, and to a large degree, elevated in individuals with ALAD-2 and higher blood Pb OR=80 (95% CI:3, 2096) in a case-control study of 63 ET cases and 101 matched controls (Louis *et al.* 2005). In a further case-control study of 106 essential tremor cases and 151 controls, Louis *et al.* (2011) found that essential tremor score was highest in individuals with higher concentrations of both Pb and harmane; authors concluded that there was an additive effect of Pb and harmane on essential tremor severity. Louis collaborated with researchers in Turkey to examine ET and Pb in Turkey, a distinct population from the New York-based data in earlier publications. In a case-control study in 105 ET patients and 105 controls from Turkey Dogu *et al.* (2007) reported that blood Pb (mean 3.2 µg/dL in cases and 1.6µg/dL in controls) was associated with a significantly greater odds ratio for ET diagnosis OR=4.19(2.59,6.78).

Summary of support for conclusions

Animal data support Pb-associated neurological effects, including tremor, at higher Pb exposure levels (see ATSDR 2007; e.g., Booze *et al.* 1983; U.S. EPA 2006 for recent reviews of the animal data). The NTP concluded that there is *limited* evidence that blood Pb levels <5µg/dL are associated with diagnosis of essential tremor because the case-control studies that reported an association with blood Pb are from only two populations and represent a small total sample size of ET patients (about 300). As with other studies of health effects of Pb in adults, prospective studies in a population for which the data demonstrated that blood Pb levels remained consistently below 10µg/dL from birth until diagnosis of ET would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL. The NTP's conclusions for *limited* evidence that blood Pb levels <5µg/dL are associated with diagnosis of ET is consistent with the blood Pb level of 3µg/dL highlighted in the two studies listed in EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead.

Parkinson's disease

There is *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL and Parkinson's disease as few studies examining this association were located and no studies were identified in groups with blood Pb levels <10µg/dL. Two studies were found that reported a significant association between lifetime Pb or bone Pb and incidence of Parkinson's disease with Pb exposure measurements. In a case-control study of 121 Parkinson's disease patients and 414 controls in Michigan, Coon *et al.* (2006) reported that the odds ratio for Parkinson's disease was increased for whole body lifetime Pb exposure (blood and bone combined by a physiologically based pharmacokinetic model) was significantly elevated in the highest Pb quartile OR=2.27(95%CI: 1.13,4.55), but there was no association with bone Pb. Weisskopf *et al.* (2010) reported that the odds ratio for Parkinson's disease was increased OR=3.21 (95%CI:1.17,8.83) for highest quartile tibia Pb to lowest in a case-control study of 330 Parkinson's disease patients and 308 controls in Boston.

4.3.4 Sensory organs

Auditory

There is *sufficient* evidence that blood Pb levels <10µg/dL in children are associated with decreased auditory acuity. Cross-sectional studies reported a Pb-related increase in hearing thresholds and an increase in latency of brainstem auditory evoked potential (BAEP) in children and young adults[6] aged 4 to 19 and in altered BAEPs in newborns (see Appendix A: Neurological Effects for full list of studies). Hearing loss indicated by higher hearing thresholds have been demonstrated at blood Pb levels <10µg/dL and increased latency of BAEP have been reported at slightly higher blood Pb levels (<10µg/dL). There is *limited* evidence that prenatal blood Pb levels <10µg/dL are associated with auditory effects because there are few studies that addressed low-level Pb exposure during this developmental period. There is also *limited* evidence that blood Pb levels <10µg/dL in adults are associated with decreased auditory acuity due to the limited number of studies with blood Pb data <10µg/dL and auditory effects in adults.

In a cross-sectional study of 4519 children and young adults 4 to 19 from the NHANES II dataset, Schwartz and Otto (1987) reported a significant association between blood Pb level and loss in hearing as determined by an increase in hearing thresholds for pure-tone frequencies from 500 to 4000 Hz. In a second study using the same measures of hearing, Schwartz and Otto (1991) found that mean hearing thresholds were significantly increased in association with blood Pb levels in a large cross-sectional study of 3545 children and young adults aged 6 to 19 from the Hispanic Health and Nutrition Survey. A loss in hearing was observed at blood Pb levels ≥8µg/dL and a 2db loss in hearing at all frequencies was reported with an increase in blood Pb from 6 to 18µg/dL (Schwartz and Otto 1991). Similar results were observed in a study of 155 Polish children aged 4-14 with a loss in hearing demonstrated by increased hearing thresholds at blood Pb levels <10µg/dL (median blood Pb of 7.2µg/dL); there were also increased latencies of peak I brainstem auditory evoked potentials (BAEP) that were significant for blood Pb levels >10µg/dL compared to children with blood Pb <4.6µg/dL (Osman *et al.* 1999). Increased latencies of BAEPs I-IV have also been reported at higher blood Pb levels (10µg/dL) in adults and children (reviewed in Otto and Fox 1993), and in two studies of children with higher mean blood Pb that included some subjects with blood Pb levels below 10µg/dL (Otto *et al.* 1985; Robinson *et al.* 1985). An effect of prenatal exposure is supported by data from the Rothenberg *et al.* (1994; 2000) studies demonstrating that the latency and interpeak interval of brainstem auditory evoked potential were significantly altered in infants (n=30 born to mothers with pregnancy Pb levels of 2.5-35µg/dL) and 5-6-year (n=100) olds born to mothers with mean blood Pb levels of 8µg/dL. Dietrich *et al.* (1992) reported that performance on the screening test for auditory processing disorders (SCAN) at 5 years of age was negatively affected in a study of 259 children from the Cincinnati Lead Study with mean prenatal blood Pb 8µg/dL, and infant blood Pb of 5µg/dL; however, mean blood Pb levels from 1-5 years of age ranged from 10 to 17µg/dL so effects may not be associated with blood Pb levels <10µg/dL.

[6] Note: children are defined as <18 years of age in this document; therefore 18 and 19 year olds in the study are considered adults

Four studies in adults addressed individuals with lower blood Pb levels. Forst *et al.* (1997) reported that blood Pb level (mean 5µg/dL; range 1 to 18) was associated with an elevated hearing threshold at 4000 Hz, but not at other frequencies examined in a study of 183 workers. Hwang *et al.* (2009) found that hearing thresholds were significantly increased in a study of 259 steel plant workers in Taiwan at blood Pb levels ≥7µg/dL (mean blood Pb =5µg/dL) for frequencies from 3000 to 8000 Hz and not for lower frequencies. A case-control study of 121 adult cases referred for hearing testing with geometric mean blood Pb 10.7µg/dL and 173 workers with normal hearing (mean blood Pb 4µg/dL) reported that blood Pb was significantly associated with higher hearing thresholds (Chuang *et al.* 2007). In cross-sectional analysis of 448 men in the Normative Aging Study (mean age 65 at time of bone Pb measurement), tibia Pb (mean 23µg/g), and patella Pb (mean 33µg/g) were significantly associated with hearing loss indicated by higher hearing thresholds at 2000 - 8000 Hz and pure-tone averages (Park *et al.* 2010). Although blood Pb and the potential relationship between blood Pb and hearing were not examined in the Park *et al.* (2010) study, other studies reported mean concurrent blood Pb levels in members of this cohort as below 10µg/dL (Rajan *et al.* 2007). Additional support for effects in adults is provided by the large cross-sectional studies of individuals from 4-19 years of age (Schwartz and Otto 1987, 1991) described earlier; these studies included young adults in the age range of the population studied (i.e., individuals aged 18 and 19 are considered adults).

Summary of support for conclusions

Animal data support a Pb-associated effect on auditory acuity determined by an increase in the latency of auditory evoked potentials at blood Pb levels higher than the level observed in human studies (i.e., 33-100µg/dL) (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The human data from several cross-sectional and prospective studies support a Pb-associated decrease in auditory acuity and increase in latency of auditory evoked potentials in children with blood Pb levels <10µg/dL. The conclusion of *sufficient* evidence for decreased auditory acuity at concurrent blood Pb levels <10µg/dL in children is based on the consistency of effects between loss of hearing as determined by increased hearing thresholds and increased latency of auditory evoked potentials. In adults, the conclusion of *limited* evidence for similar effects at concurrent blood Pb levels <10µg/dL is due to the small number of studies supporting an effect (two at blood Pb <10 with total n less than 450), the two Schwartz and Otto (1987, 1991) cross-sectional studies that included adults age 18 and 19 in studies primarily focused on children and supporting evidence from occupational studies at higher blood Pb levels (e.g., Bleecker *et al.* 2003), and additional supporting evidence of an effect of Pb from bone Pb data in a population of elderly men. As with other studies of health effects of Pb in adults, prospective studies in a population for which the data demonstrated that blood Pb levels remained consistently below 10µg/dL from birth until measurement of auditory acuity would eliminate the potential role of early-life blood Pb levels above 10µg/dL on auditory effects observed in adults with concurrent blood Pb levels <10µg/dL. The conclusion of *limited* evidence for prenatal exposure to blood Pb <10µg/dL are associated with auditory effects is based on the two Rothenberg *et al.* (1994; 2000) studies and the Dietrich *et al.* (1992) study that demonstrated an effect of maternal exposure, but only provided data on 100 individuals with blood Pb <10µg/dL that remained below 10µg/dL until auditory function was tested. The NTP's conclusions for *sufficient* evidence that blood Pb levels <10µg/dL are associated with

decreased auditory acuity in children, are in line with the supportive evidence of a relationship with auditory processing decrements outlined in EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead.

Visual

There is *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL and effects on vision in children or adults. Multiple studies reported an inverse relationship between blood Pb levels at and below 10µg/dL and visual-motor performance evaluated with tests such as the Beery Developmental Test (e.g., Al-Saleh *et al.* 2001; Chiodo *et al.* 2004; Wasserman *et al.* 2000); however, few epidemiological studies addressed the effects of low-level Pb exposure on visual function. Only three studies were located that examined the impact of blood Pb levels <10µg/dL and retinal or visual function. Maternal blood Pb at 12 weeks of pregnancy (mean 8.5µg/dL) in 45 participants of the Mexico City Prospective study was associated with altered retinal function in 7-10-year-old children indicated by changes in electroreinographic (ERG) testing results (Rothenberg *et al.* 2002). In a study of 100-200 children in Artic Quebec at 5 and 11 years of age, Boucher *et al.* (2009) demonstrated that cord blood Pb (mean 5µg/dL) was significantly associated with changes in event-related potential P3b wave amplitude at 5 years, but not in 11-year olds. Altmann *et al.* (1998) reported that blood Pb levels (mean 4µg/dL) in 6-year-old children (n= 384) in Germany were associated with altered visual function as determined by changes in visual-evoked potential (VEP) interpeak latency. Review of animal data includes evidence for retinal and visual cortical structural and functional abnormalities in rats and non-human primates at 11-300µg/dL (reviewed in ATSDR 2007; Fox and Boyles 2007; Otto and Fox 1993; U.S. EPA 2006). Recent studies in rats support the limited data in humans, and rats exposed to Pb also displayed significant changes in electroreinographic (ERG) testing results (Fox *et al.* 2008). The NTP concludes that there is *inadequate* evidence to evaluate the potential association between blood Pb <10µg/dL because of the general lack of human data on retinal or visual function in individuals with blood Pb levels <10µg/dL. Increased latency in the visual evoked potential has been demonstrated in studies of adults with higher blood Pb levels (e.g., 60 down to 17µg/dL in Abbate *et al.* 1995). The report of a potential lower threshold of 14µg/dL for postural sway in adults with higher occupational Pb exposure (Iwata *et al.* 2005) provides some support for an effect on the auditory and visual systems, because postural sway requires the integration of visual and vestibular input along with peripheral sensory input and motor output. The NTP's conclusions for *inadequate* evidence that blood Pb levels <10µg/dL are associated with effects on vision in humans are in line with the supportive evidence of a relationship with auditory processing decrements outlined in EPA's 2006 AQCD for Lead and ATSDR's 2007 Toxicological Profile for Lead and identification of a potential threshold of 14-20µg/dL for effects including visual evoked potentials in adults.

4.4 Conclusions

NTP concludes there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with neurological effects in children and *limited* evidence in adults (see **Table 4.3: NTP conclusions on neurological effects of low-level Pb** for complete list of conclusions). A major strength of

the evidence for effects of low-level Pb on neurological outcomes is in the consistency of results for an adverse effect of blood Pb <10µg/dL across multiple indices of neurological effects (e.g., cognition, behavior and sensory function), through multiple populations, a wide age range from early childhood to older adults, and from studies with substantial methodological heterogeneity. In some studies, blood Pb levels of 2µg/dL are associated with effects in children (ADHD in Cho *et al.* 2010; e.g., academic achievement in Miranda *et al.* 2007). In children, there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with various indices of reduced cognitive function, increased incidence of ADHD diagnosis, increased behavioral problems and *sufficient* evidence that blood Pb levels of 10µg/dL are associated with lower IQ and decreased auditory function. In adults, there is *limited* evidence that blood Pb levels <10µg/dL are associated with psychiatric outcomes including anxiety and depression, decreased auditory function, decreases in specific measures of cognitive function in older adults, and neurodegenerative diseases including ALS and essential tremor. There are more consistent associations between bone Pb than blood Pb and decreases in cognitive function in older adults, suggesting a role for cumulative Pb exposure in Pb-related cognitive decline.

Table 4.3: NTP conclusions on neurological effects of low-level Pb				
Health Effect	**Population or Exposure Window**	**NTP Conclusion**	**Blood Pb Evidence**	**Bone Pb Evidence**
Cognitive Function: Academic Achievement	Prenatal	*Inadequate*	No studies located	Not studied
	Children	*Sufficient*	Yes, <5µg/dL	Yes, tooth dentin Pb
Cognitive Function: IQ	Prenatal	*Limited*	Yes, <10µg/dL	Not studied
	Children	*Sufficient*	Yes, <10µg/dL	Yes, tibia and tooth dentin Pb
		Limited	Yes, <5µg/dL (one study)	
Cognitive Function: Other general and specific measures	Prenatal	*Limited*	Yes, <5µg/dL	Not studied
	Children	*Sufficient*	Yes, <5µg/dL	Yes, tibia and tooth dentin Pb
	Older adults	*Limited*	Yes, <10µg/dL	Yes, tibia and patella Pb
Behavior -ADHD	Prenatal	*Limited*	Yes, <10µg/dL	Not studied
	Children	*Sufficient*	Yes, <5µg/dL	Yes, tibia and tooth dentin Pb
	Adults	*Inadequate*	No studied located	Not studied
Behavior-Problems	Prenatal	*Limited*	Yes, <10µg/dL	Not studied
	Children	*Sufficient*	Yes, <5µg/dL	Yes tooth dentin Pb, bone, hair
	Adults	*Inadequate*	No studies located	Not studied
Psychological effects: Depression, Anxiety, other	Prenatal	*Inadequate*	No studies located	Not studied
	Children	*Inadequate*	Unclear, some data >10µg/dL	Not studied
	Adults	*Limited*	Yes, <10µg/dL	Tibia and patella Pb
NeuroDegeneration: ALS	Adults	*Limited*	Yes, <10µg/dL	Yes, tibia and patella
ND: Alzheimer's disease	Adults	*Inadequate*	No studies <10µg/dL located	Not studied
ND: Essential Tremor	Adults	*Limited*	Yes, <10µg/dL	Not studied
ND: Parkinson's disease	Adults	*Inadequate*	No studies <10µg/dL located	Yes, tibia and PBPK -cumulative
Sensory Function: Auditory	Prenatal	*Limited*	Yes, <10µg/dL	Not studied
	Children	*Sufficient*	Yes, <10µg/dL	Not studied
	Adults	*Limited*	Yes, <10µg/dL	Yes, tibia and patella
Sensory Function: Visual	Prenatal	*Inadequate*	No studies located	Not studied
	Children	*Inadequate*	Yes, <10µg/dL	Not studied
	Adults	*Inadequate*	No studies <10µg/dL located	Not studied

Notes: ADHD – attention deficit hyperactivity disorder; ALS – amyotrophic lateral sclerosis

5.0 IMMUNE EFFECTS

5.1 Conclusions:

The NTP concludes that there is *limited* evidence that blood Pb levels <10µg/dL are associated with adverse immune effects in children and that there is *inadequate* evidence in adults.

In children, there is *limited* evidence that blood Pb levels <10µg/dl are associated with increased hypersensitivity responses and allergic sensitization diagnosed by skin prick testing to common allergens. There is *sufficient* evidence that blood Pb levels of 10µg/dL and below are associated with elevated serum IgE in children up to the age of 17. Six studies with mean blood Pb levels of 10µg/dL and below support the relationship between blood Pb and increased serum IgE (see **Table 5.3**). Although increases in serum levels of total IgE do not equate to disease, elevated levels of IgE are the primary mediators of type-I hypersensitivity associated with allergic sensitization and the data demonstrating Pb-related increases in IgE support an association with hypersensitivity. Further support of an association between blood Pb levels <10µg/dL and hypersensitivity is provided by a prospective study on Pb-related increased allergic sensitization demonstrated by positive response to skin prick testing to common allergens. Together these data support the conclusion of *limited* evidence that blood Pb levels <10µg/dL are associated with increased hypersensitivity. However, there is *inadequate* evidence of an association between blood Pb and other allergic diseases such as eczema or asthma.

There is *inadequate* evidence in adults to address the potential association between blood Pb <10µg/dL and IgE, allergy, eczema, or asthma. Few studies have investigated the relationship between immune function and lead in adults or children, and most studies report general observational markers of immunity rather than function. There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with observational immune endpoints such as altered lymphocyte counts or serum levels of IgG, IgM or IgA in the peripheral blood of children or adults because of a general lack of studies at the lower dose and inconsistency in available data. There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with changes in immune function other than hypersensitivity because there are few studies of Pb and immune function in humans, particularly at lower blood Pb levels. Very few studies examine markers of exposure other than blood Pb levels, and therefore it is unknown if blood or bone Pb levels would be a better indicator of immune effects.

5.2 How conclusions were reached

Conclusions in the NTP's evaluation of Pb-related immunological effects in humans associated with low-level Pb are derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10µg/dL. Although there is a large database of immune studies of Pb in laboratory animals, the database of human studies is somewhat limited, particularly at blood Pb levels <10µg/dL. The NTP's conclusions are based on the evidence from human studies with blood Pb levels of <10µg/dL with data reflecting exposure levels up to 15µg/dL also considered

so that effects at and around 10µg/dL were not excluded from the evaluation. Given the limited database of human studies available to evaluate immune effects associated with blood Pb levels <10µg/dL, a discussion of immune effects associated with higher blood Pb levels is also included in the evaluation. The discussion below also assesses the biological plausibility and support for Pb–associated immune effects provided by the database of studies in laboratory animals. Major endpoints considered as potential indicators of effects of Pb on the immune system are listed and briefly described in **Section 5.2.1**. This document is not a review of the immune system or immunotoxicity and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP's conclusions are discussed in detail in **Section 5.3 Evidence for Pb-related Immune Effects.** The discussion of each immune effect begins with a statement of the NTP's conclusion that the specific effect is associated with a blood Pb level <10µg/dL or <5µg/dL and the age group in which it is identified (childhood or adulthood) as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. Although the information necessary to support the NTP's conclusions is presented in **Section 5.3**, the complete dataset of human studies considered for evaluation of immune effects with low-level Pb is included in Appendix B: Immune Effects and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 5.2.2** below.

5.2.1 Principal Measures of Immune Effects

Table 5.1 lists a number of key immune endpoints potentially evaluated in epidemiological studies. A distinction is made between observational markers and which generally have less predictive value for immunotoxicity and markers of immune function which are a stronger indicator of potential adverse immune effects. Functional assays can be performed in humans including specific antibody response to vaccination, delayed type hypersensitivity (DTH) response, phagocytic activity of neutrophils and macrophages, oxidative burst of neutrophils and macrophages (Tryphonas 2001). However,

Table 5.1: Major immune effects considered

Effect	Description
Observational:	
Immunoglobulin (Ig) or antibodies	Serum IgE, IgM, IgG; IgA and IgD (A and D not routinely measured)
Immunophenotyping	White blood cell differential (T-cells, B-cells, NK-cells, monocytes/macrophages, etc.)
Functional:	
Antibody response	Production of Ig to challenge: **Hypersensitivity** evaluated with antigen-specific IgE and skin prick test (SPT) **Suppression** commonly evaluated by specific IgM or IgG following T-cell antigen challenge
DTH response	Th-1 and macrophage dependent Delayed-type Hypersensitivity response to antigen challenge
Neutrophils	PMN phagocytosis, respiratory burst, migration
Monocyte/ macrophages	Phagocytosis, respiratory burst (monocytes circulate and mature into tissue macrophages)
Allergy, asthma, eczema, etc.	Clinical manifestation of hypersensitivity

human epidemiological data are much more likely to be restricted to observational data such as circulating immunoglobulin levels, lymphocyte counts, and cytokine levels. The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 5.3** below.

5.2.2 Principal conclusions from 2006 EPA and 2007 ATSDR Pb documents:

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both list a number of immune parameters (see **Table 5.2** for principal conclusions and original documents for complete conclusions) that have been reported as altered in populations exposed to Pb including: increased serum IgE levels, altered T-cell and B-cell numbers, changes in macrophage and neutrophil activation, suppressed neutrophil chemotaxis and phagocytosis. The 2006 EPA AQCD for Lead states that studies have consistently found consistent evidence of increased serum IgE levels in children at blood Pb <10µg/dL, but that results from studies in adults are mixed. The 2006 EPA AQCD for Lead also states that the principal functional immune changes associated with Pb exposure are: 1) increasing the type 2 helper T-cell (Th-2)-associated production of IgE; 2) suppressed type 1 helper T-cell (Th-1) responses (i.e., delayed type hypersensitivity or DTH); 3) shifting the balance of Th-1/Th-2 cytokines toward a Th-2 response and; 4) stimulating macrophages into a hyper-inflammatory state. EPA notes that functional changes had not been rigorously evaluated in human studies at the time of the 2006 AQCD for Lead, and that the available epidemiological studies rely primarily on observational data detailing circulating immunoglobulin levels and lymphocyte counts. EPA is in the process of revising the AQCD, and the conclusions of the external draft (U.S. EPA 2011) are largely in line with the 2006 AQCD for Lead.

Table 5.2: Main conclusion for immunological effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"…effects include changes in serum immunoglobulin levels; perturbation of peripheral lymphocyte phenotype profiles, including decreases in peripheral blood T-cell abundance and changes in T-cell to B-cell abundance ratios; suppression of lymphocyte activation; and suppression of neutrophil chemotaxis and phagocytosis. Studies of biomarkers of humoral immunity in children have consistently found significant associations between increasing blood Pb concentrations and serum IgE levels at blood Pb levels <10 µg/dL " (EPA, 2006 pg 6-272)
"Altered immune parameters have been described in lead workers with PbB in the range of 30–70 µg/dL. Reported effects included changes in some T-cell subpopulations, response to T-cell mitogens, and reduced chemotaxis of polymorphonuclear leukocytes. Several studies of children reported significant associations between PbB and increases in serum IgE levels… "(ATSDR, 2007 pg 22)

The NTP considered the conclusions and data summaries from the EPA and ATSDR documents. In general, the NTP concurred with the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints in the document.

5.3 Evidence for Pb-related Immune Effects

5.3.1 Increased Serum IgE and Allergic Sensitization

There is *sufficient* evidence that blood Pb levels at and below 10µg/dL are associated with increased serum IgE in children up to age 17 (**Table 5.3** and Appendix B: Immune Effects). Elevated serum IgE was reported with Pb exposure in 6 cross-sectional studies involving children from multiple populations in North America, Europe and China. A positive association between blood Pb and IgE has been observed in children at mean blood Pb values from 1.9µg/dL to 14µg/dL and subjects ranging in age from 1 month to 17 years of age. The database for potential association between blood Pb and IgE in children is restricted to cross-sectional studies and therefore the data only inform the association between current blood Pb and current IgE. The influence of prenatal Pb exposure on IgE was the subject of a single study (Annesi-Maesano *et al.* 2003) that demonstrated a positive association between IgE and infant hair Pb levels, but provided equivocal data on the association between maternal blood Pb and infant IgE at birth. Although increases in serum levels of total IgE do not equate to disease, elevated IgE is the primary mediator of type-I hypersensitivity associated with allergic sensitization and allergic disease such as asthma (Ahmad Al Obaidi *et al.* 2008; Beeh *et al.* 2000). The data supporting Pb-related increases in IgE, together with a prospective study on allergic sensitization (diagnosed by skin prick testing), provide *limited* evidence that blood Pb <10µg/dL is associated with increased hypersensitivity responses in children. However, there is *inadequate* evidence of an association between blood Pb and eczema or asthma in children because the results of the few available studies are generally negative for asthma and the eczema data come from a pair of studies of a single population of children from a dermatology clinic. There are fewer studies of the association between low level lead (blood <10µg/dL) and hypersensitivity in adults than for children, and available data that address IgE and related endpoints are mixed. There is *inadequate* evidence in adults to address the potential association between blood Pb <10µg/dL and IgE, allergy, eczema, or asthma.

Increased serum IgE was reported in five cross-sectional studies of children from 1 month to 17 years of age and mean blood Pb from 1.9µg/dL to 14µg/dL (Hon *et al.* 2009; Hon *et al.* 2010; Hon 2011; Karmaus *et al.* 2005; Lutz *et al.* 1999; Sun *et al.* 2003). Serum IgE was positively correlated (r=0.22; p=0.0004) with blood Pb levels in a study of 279 children in Missouri aged 9 months to 6 years (Lutz *et al.* 1999). Serum IgE was also correlated with blood Pb levels (r=0.48; p=0.002) in a subsample of 72 children from a larger study of 217 children aged 3-6 with mean blood Pb of 9.5µg/dL in China (Sun *et al.* 2003). Although the Lutz *et al.* (1992) and Sun *et al.* (2003) studies report an effect of blood Pb at levels near 10µg/dL; additional studies demonstrate increased IgE at mean blood Pb levels in the range of 2µg/dL. Serum IgE was positively correlated with blood Pb (mean = 1.9µg/dL) in 110 children with eczema in which the age ranged from 1 month to 17 years for the participants recruited from a dermatology clinic in Hong Kong (Hon *et al.* 2009; Hon *et al.* 2010; Hon 2011). Karmaus *et al.* (2005) reported increased serum IgE in a study of 331 children aged 7-10 in Germany at blood Pb levels above 2.8µg/dL, the median for the population.

Table 5.3: Studies of serum IgE, sensitization, and eczema with low level Pb used to develop conclusions for children				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Immunological Findings**	**Reference**
Increased IgE				
Supporting	374 newborns in Paris Study A – 1985 Study B – 1991-1992	Cross-sectional	**Increased serum IgE** in cord blood was associated with infant hair levels of Pb. IgE was also associated with maternal blood Pb in study B (Pb=6µg/dL) not study A (Pb=13µg/dL), or infant blood in either study.	Annesi-Maesano (2003)
Supporting	331 children aged 7-10 in Germany	Cross-sectional	**Increased serum IgE** was associated with current blood Pb (mean Pb 2.7µg/dL).	Karmaus (2005)
Equivocal	318 children aged 0.5-7 in Egypt	Cross-sectional	**Serum IgE** differed by blood Pb (mean Pb 9.2µg/dL); however authors report that IgE is not correlated with blood Pb	Hegazy (2011)
Supporting	279 children aged 0.75-6 in Missouri	Cross-sectional	**Increased serum IgE** was associated with current blood Pb (mean not reported, 64% had blood Pb<10µg/dL).	Lutz (1999)
Supporting	72 children aged 3-6 in China of 217 in study	Cross-sectional	**Increased serum IgE** was correlated with blood Pb in children with Pb≥10µg/dL. Increased serum IgE in girls with blood Pb≥10µg/dL relative to Pb<10µg/dL; not boys.	Sun (2003)
Hong Kong Eczema patents		Cross-sectional	**Increased serum IgE** was positively correlated with blood Pb (mean Pb 1.9µg/dL) in children with eczema; age > 1 month, mean age= 10	Hon (2010); may overlap with Hon 2009
Supporting	110 children age ≤17			
Supporting	58 children age 10	Cross-sectional	**Increased serum IgE** was positively correlated with blood Pb (mean Pb 1.9µg/dL) in children with eczema; age > 1 month, authors state average age of 10 years	Hon (2009) *Also for eczema*
Supporting	2470 children aged 5-14 in Germany	Ecological	**Odds ratio for increase in specific IgE** to common allergens were elevated in children from a polluted area in Germany that had higher Pb dustfall; no blood Pb data.	Heinrich (1999) *Also for sens. & eczema*
Evidence of enhanced sensitization based on skin prick test (SPT)				
Supporting	224 children aged 5 in Poland	Prospective	Frequency of atopy (positive SPT) associated with cord Pb (mean 1.2µg/dL) & maternal blood Pb (mean 1.6µg/dL); not current Pb. Risk ratio for SPT/atopy related to cord Pb; authors state that prenatal Pb may enhance sensitization to common aeroallergens.	Jedrychowski (2011)
Supporting	2470 children aged 5-14 in Germany	Ecological	Odds ratio for sensitization (positive SPT) or allergy (doctor diagnosis) was elevated in children from a polluted area in Germany that had higher Pb dustfall; no blood Pb data.	Heinrich (1999) *Also for IgE and eczema.*
Eczema and atopic dermatitis				
Not supporting	1768 children born in Boston 1979-1981	Retrospective	Relative risk of eczema in childhood (age not reported) did not differ for children with cord blood Pb>10µg/dL compared to rest of population.	Rabinowitz (1990)
Hong Kong Eczema patents		Cross-sectional	Atopic dermatitis severity, eczema severity score, and children's dermatology life quality index were positively correlated with blood Pb (mean 1.9µg/dL).	Hon (2010); may overlap with Hon 2009
Supporting	110 children age ≤17			
Supporting	58 children age 10	Cross-sectional	Atopic dermatitis severity, eczema severity score, and children's dermatology life quality index were positively correlated with blood Pb (mean 1.9µg/dL), mean age 10.	Hon (2009) *Also for IgE*
Supporting	2470 children aged 5-14 in Germany	Ecological	Odds ratio for eczema was elevated in children from a polluted area in Germany that also had higher Pb dustfall; no blood Pb data.	Heinrich (1999) *Also for IgE and sens.*

* Epidemiological studies of low level Pb exposure, Immunoglobulin E (IgE), sensitization and eczema listed by decreasing cohort size and grouped together for overlapping study populations. Blood Pb levels up to 15 µg/dL were included so that effects at and around 10µg/dL were not excluded from the evaluation.

There is some evidence that in children the association of IgE with blood Pb may exhibit a nonmonotonic dose response with increased IgE reported at blood Pb levels from 2 to 20 µg/dL and decreasing serum IgE at higher blood Pb levels (i.e., >20µg/dL). Nonmonotonic dose responses are thought to reflect multiple mechanisms of toxicant action affecting a given endpoint (including immune effects) differently at different doses (Welshons *et al.* 2003). For example, Narita *et al.* (2007) described stimulation of IgE-mediated release of allergic mediators from human mast cells with surface bound IgE in response to toxicants including Aroclor 1242; a nonmonotonic dose response was reported for IgE-mediated release of β-hexosaminidase with lower doses resulting in activation of this key step in allergic reactions and higher doses having no effect. Blood Pb levels above 20µg/dL (23-42µg/dL determined graphically from Wagnerova *et al.* 1986) were associated with decreased IgE in a study of 11 year old children in Czechoslovakia in which both the exposed and referent population had blood Pb levels above 10µg/dL (Wagnerova *et al.* 1986). In the Lutz *et al.* (1999) study described earlier, serum IgE differed significantly ($p<0.05$ Kruskal-Wallis) by blood Pb levels stratified by CDC blood Pb classification levels (I=<10; IIA=10-14; IIB=15-19; III=20-44µg/dL); however IgE was not increased in the group with blood Pb levels above 20µg/dL (i.e., IgE=52 IU/ml at blood Pb<10µg/dL; IgE=74 IU/ml at blood Pb10-14µg/dL; IgE=210 IU/ml at blood Pb 15-19µg/dL; IgE=64 IU/ml at blood Pb 20-44µg/dL). In a similar analysis of 318 children in Egypt under 8 years of age, serum IgE differed also significantly ($p=0.001$ Kruskal-Wallis) by blood Pb levels stratified by CDC blood Pb classification levels (IA=<5; IB=5-9; IIA=10-14; IIB=15-19; III=20-44; IV=45-69 µg/dL); however, the correlation between blood Pb and IgE was not significant ($p=0.12$) for the overall population with mean blood Pb of 9.2µg/dL (Hegazy *et al.* 2011). The reason for the lack of a significant correlation with blood Pb in the Hegazy *et al.* (2011) study is not clear, but it may relate to differential effects of Pb at low and high blood Pb levels.

Prospective studies are not available to examine the relationship between blood Pb and serum IgE at later time points in children. However, in a study of newborns in Paris, Annesi-Maesano *et al.* (2003) demonstrated a positive association between cord IgE and infant hair Pb ($p<0.001$). Maternal blood Pb and infant blood Pb were not correlated to cord IgE levels, although the authors report that an association between maternal blood Pb and cord IgE was at borderline significance ($p<0.1$) (Annesi-Maesano *et al.* 2003). As discussed in **Section 3.2**, hair Pb has been examined in a number of studies because collection is easy and minimally invasive; however, an ATSDR expert panel concluded that widespread use is not recommended due to unresolved scientific issues in collection and analysis (ATSDR 2001).

As discussed above, elevated levels of total IgE are associated with allergic sensitization and allergic disease such as asthma (Ahmad Al Obaidi *et al.* 2008; Beeh *et al.* 2000; Kotaniemi-Syrjanen *et al.* 2002). However, there is *limited* evidence that blood Pb levels <10µg/dL are associated with increased incidence of allergic sensitization in children. This conclusion is based two lines of evidence: 1) a prospective study reporting a significant association between maternal or cord blood Pb below 10µg/dL and greater incidence of sensitization to common allergens in children, and 2) the data supporting Pb-associated increases in serum IgE in children. In a prospective study of the children of 224 women in Poland recruited in the 2nd trimester, allergic sensitization or atopy was determined by skin prick test to common allergens

administered when the children were 5 years old (Jedrychowski *et al.* 2011). Frequency of sensitization was significantly associated with maternal blood Pb (p=0.006; mean Pb= 1.6µg/dL) and with cord blood Pb (p=0.001; mean Pb= 1.2µg/dL), but not with current blood Pb levels in the 5 year olds (p=0.43; mean Pb= 2.0µg/dL). In an analysis of the relative risk, cord blood Pb in the Jedrychowski *et al.* (2011) study was associated with an increased relative risk (RR=2.28 ([95%CI:1.1,4.6)) of atopy as indicated by at least one positive skin prick test. The 224 individuals included in the statistical analysis all had cord blood Pb levels <2.5µg/dL as the authors state that outliers above the 95 percentile were removed prior to analysis. The effect of the removal of individuals with higher blood Pb levels is unknown. No other studies were located that reported blood Pb and sensitization; however there is an ecological study that supports the positive association between Pb exposure and sensitization in children. The odds ratio for positive skin prick test (OR=1.38 (95%CI:1.02, 1.86)) and increased specific IgE (OR=1.75 (95%CI:1.31,2.33)) to common allergens were also elevated in children from an area in Germany with higher Pb dustfall and Pb emissions. The ecological study of 2470 children aged 5-14 lacked blood Pb data and compared children from areas with high Pb dustfall to referents (Heinrich *et al.* 1999).

There are few studies of eczema or atopic dermatitis in children and the evidence of an association with blood Pb are restricted to a single group of 110 children at a dermatology clinic and therefore may represent a sensitive subpopulation. In two overlapping studies of 110 patients with eczema from a Hong Kong pediatric dermatology clinic, blood Pb was significantly associated with atopic dermatitis severity, eczema severity score, children's dermatology life quality index and eosinophil count (Hon *et al.* 2009; Hon *et al.* 2010; Hon 2011). The association between blood Pb and clinical diagnosis for severity among the eczema patients is supported by the objective measure of a Pb-related increase in eosinophil count (r=0.27; p=0.001). The Hon *et al.* (2009; 2010; 2011) studies report an association between blood Pb and multiple clinical parameters rating atopic dermatitis severity of symptoms, not the incidence of eczema. Blood Pb levels did not differ between 110 eczema patients and 41 patients at the dermatology clinic with other skin conditions that did not have eczema (p=0.160); however the study did not have a non-atopic reference population. The Heinrich *et al.* (1999) ecological study described earlier also reported an increased odds ratio for eczema (OR=1.52 (95%CI:1.03, 2.24)) among children living in the area with higher Pb dustfall. However, a retrospective study of 1768 children born in Boston between 1979-1981 that determined relative risk of eczema in childhood with cord blood Pb levels, did not find a difference between children with cord blood Pb levels above and below 10µg/dL (Rabinowitz *et al.* 1990). This study differs from the two Hon *et al.* (2009; 2010) studies in the timing of the Pb exposure measurement and in the reporting of incidence rather than severity of eczema. The Rabinowitz *et al.* (1990) study compared cord blood Pb to eczema years later (exact timing not reported), and addressed incidence rather than severity of eczema.

Although four retrospective studies examined the potential relationship between Pb exposure and asthma in children, the results are primarily negative and only one of the four studies reported an association between asthma and blood Pb. Blood Pb levels >10µg/dL were associated with an increased odds ratio for doctor diagnosis of asthma

(OR=7.5(95%CI:1.3,42.9)) in a study of 356 children under the age of 13 in the STELLAR database in Michigan (Pugh Smith and Nriagu 2011). The analysis in Pugh Smith *et al.* (2011) was thoroughly adjusted for risk factors associated with asthma or confounders related to Pb exposure such as age, gender, or exposure to passive smoke, cats, dogs, cockroaches or other factors known to contribute to asthma. A retrospective study of 4634 children in managed care in Michigan did not find an association between blood Pb at 1-3 years of age and asthma incident based on insurance records or medication dispensing events in an analysis adjusted for income, birth weight and sex (Joseph *et al.* 2005). A retrospective study of 1768 children born in Boston between 1979-1981 that determined relative risk of asthma in childhood with cord blood Pb levels, did not find a difference between children with cord blood Pb levels above and below 10µg/dL in an analysis that did not include adjustment for confounders (Rabinowitz *et al.* 1990). Myers *et al.* (2002) reported that the incidence of asthma based on medical records did not differ between 151 patients in Chicago with high blood Pb levels (≥25µg/dL) and referents with blood Pb<5µg/dL. The Myers *et al.* (2002) tested for effects of high blood levels and did not report any adjustments for confounders.

The data from Sun *et al.* (2003) in girls and Pizent *et al.* (2008) in women suggest that Pb exposure may have a stronger effect on IgE in females. The results also indicate that analyses of Pb and IgE should consider sex as a potential confounder; which is not unexpected because sex differences in serum IgE are apparent early in childhood (Hunninghake *et al.* 2011) and other toxicant-induced or exacerbated hypersensitivity reactions have a gender bias (Corsini and Kimber 2007). All of the studies of IgE adjust for age, except Hon (Hon *et al.* 2009; Hon *et al.* 2010). Smoking or exposure to passive smoke has also been associated with IgE. The effects of smoke exposure may be two-fold, as exposure may increase IgE directly or it may lead to increased exposure to Pb because of the Pb content in tobacco smoke. In a study of 318 children in Egypt under 8 years of age, Hegazy *et al.* (2011) reported a significant correlation ($r=0.133$; $p<0.05$) between blood Pb and parental tobacco smoking. The analysis of IgE in the Karmaus *et al.* (2005) study was particularly thorough in its consideration and adjustment for confounders including gender, age, number of infections in the last 12 months, exposure to passive smoke, and exposure to other toxicants including DDE (which was also associated with increased IgE).

In adults, the results are mixed for an association between blood Pb and IgE or sensitization-related endpoints, but there are only three relevant studies in adults with blood Pb levels below 10µg/dL: a study of 523 office workers in Korea (Min *et al.* 2008), a study of 216 office workers in Croatia (Pizent *et al.* 2008), and a study of 94 Italians without occupational exposure to Pb (Boscolo *et al.* 1999; Boscolo *et al.* 2000). There was no correlation between blood Pb levels and serum IgE in men or women in the Boscolo *et al.* (1999; 2000) publications. Serum IgE was correlated to blood Pb levels in the female office workers in the Pizent *et al.* (2008) study, but not in the men. The Pizent *et al.* (2008) study also examined functional endpoints (sensitization to allergens by positive skin prick test and non-specific bronchial reactivity by histamine challenge) as well as the observational data on serum IgE. Although Pb (range 0.56-7µg/dL) exposure was associated with increased IgE in women, there was no effect of blood Pb on sensitization or bronchial reactivity. In men, blood Pb (range 1-7µg/dL) was not associated with

serum IgE, and blood Pb was associated with decreased hypersensitivity responses including decrease in sensitization to allergens and decrease in non-specific bronchial reactivity (Pizent *et al.* 2008). In contrast, Min *et al.* (2008) reported a significant positive association between blood Pb (mean 3µg/dL) and non-specific bronchial reactivity (by methacholine bronco-provocation test) in both male and female office workers. There are two additional studies of IgE in adults that support an association with Pb at higher blood Pb levels. Serum IgE was positively correlated with blood Pb levels in two studies: a study of 47 Pb refinery workers in Osaka with mean blood Pb 50µg/dL (Horiguchi *et al.* 1992) and a study of 606 Pb battery workers in Korea with mean blood Pb 23µg/dL in which IgE was elevated in workers with blood Pb above 30µg/dL relative to workers with blood Pb <30 (Heo *et al.* 2004). Collectively, there are only 4 studies that report data on blood Pb and IgE in adults. The two high exposure studies (mean blood Pb levels of 23 and 50µg/dL) suggest there may be an association between IgE and blood Pb ≥30µg/dL or even higher (Heo *et al.* 2004; Horiguchi *et al.* 1992); the two studies with blood Pb levels below 10µg/dL are negative for effects in men and report mixed results in women (Boscolo *et al.* 1999; Boscolo *et al.* 2000; Pizent *et al.* 2008). An increase in symptoms of asthma and rhinitis was also reported in male industrial workers in the United Arab Emirates with extremely high blood Pb levels (mean of 78µg/dL) relative to referents that also had high blood Pb levels (20µg/dL) (Bener *et al.* 2001). In adults with blood Pb levels <10µg/dL, the data for asthma or respiratory symptoms are conflicting, with one study reporting increased bronchial responsiveness (Min *et al.* 2008) and one study reporting decreased bronchial responsiveness or no effect of Pb (Pizent *et al.* 2008). The data for sensitization with blood Pb are negative, with no effect in female office workers and reduced sensitization with increasing blood Pb in males, although data are from a single study (Pizent *et al.* 2008).

Summary of support for conclusions

Animal data support an increase in IgE in adult mice at high Pb levels (50µg s.c. 3x week for 3 weeks) and associated with developmental Pb exposure at levels that include blood Pb <10µg/dL (2-20µg/dL in mice and 40µg/dL in rats; seeATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). Prenatal or postnatal exposure to Pb at low, environmentally relevant levels in mice have been associated with increased IgE at later time points. Plasma IgE levels were significantly increased in neonatal BALB/c mice with blood Pb levels from 2-20µg/dL at 2 weeks of age in mice that were the offspring of mothers exposed to Pb in drinking water during gestation alone, lactation alone, or during both periods (unexposed neonate blood Pb was approximately 3 µg/dL and maternal blood Pb not reported Snyder *et al.* 2000). A number of studies in mice also support Pb-associated skewing of T-cell response toward a type 2 helper T-cell (Th-2) response, which is associated with allergy and increased IgE production, versus a type 1 helper T-cell (Th-1) response which is associated with host resistance and delayed type hypersensitivity (reviewed in Dietert and Piepenbrink 2006; U.S. EPA 2006). The human data supporting a Pb-associated increase in serum IgE in children are restricted to cross-sectional studies. The determination of causation from cross-sectional studies has the inherent limitation of making conclusions based on current blood Pb measurements and lacking information on cumulative Pb or Pb exposure at earlier time points that may be associated with the mechanisms to elevate serum IgE. The demonstration that infant hair Pb in newborns was associated with cord IgE levels from the Annesi-Maesano *et al.* (2003) study suggests that Pb

exposure at earlier time points is associated with IgE in children, but prospective studies are lacking. The most relevant time for measuring exposure relative to IgE may include both earlier time points relating to mechanisms of Th-2 skewing (Parronchi *et al.* 2000) or current blood Pb directly related to secretion of IgE because IgE in serum has a half-life of less than 2 days. The conclusion of *sufficient* evidence that blood Pb <10µg/dL are associated with elevated serum IgE in children is based on the 6 cross sectional studies that report a correlation between blood Pb (mean levels from 2 to 10µg/dL) and serum IgE in children up to age 17. Elevated levels of serum IgE were reported in children with increasing blood Pb across multiple studies from different populations in analyses that adjusted or controlled for age, sex, and in some cases smoking and exposure to other contaminants known to effect serum IgE. The conclusion of *limited* evidence for increased hypersensitivity responses at blood Pb <10µg/dL in children is supported by the demonstration of Pb-related increases in IgE together with the Jedrychowski *et al.* (2011) prospective study on allergic sensitization (diagnosed by skin prick testing). There are some data supporting an association between blood Pb<10µg/dL and eczema or asthma in children; however the conclusion of *inadequate* evidence is because the eczema studies with blood Pb are from a single patient population and represent 110 total children and the data supporting an association with asthma are restricted to a single study. For adults, there are only three studies of IgE or sensitization related effects of people with blood Pb levels <10µg/dL. There is *inadequate* evidence in adults to address the potential association between blood Pb <10µg/dL and IgE, allergy, eczema, or asthma. The NTP's conclusions for *sufficient* evidence for increased serum IgE in children at blood Pb levels <10µg/dL are in line with the conclusion of a consistent association from the 2006 EPA AQCD for Lead and significant associations in ATSDR's Toxicological Profile for Lead.

5.3.2 IgG, IgM and Antibody Response

There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with serum levels of IgG, IgM or IgA in the peripheral blood of children or adults (see Appendix B: Immune Effects). In studies that examined serum immunoglobulins, there is no evidence of a consistent change, either increase or decrease associated with blood Pb below or above 10µg/dL. There is *inadequate* evidence that blood Pb at any level is associated with changes to the IgM or IgG specific functional antibody response. Two studies were located that evaluated an antibody response in humans in conjunction with blood Pb levels, and both reported that there was no effect of blood Pb level on the antibody response. There was no effect of blood Pb levels on the anti-rubella IgG antibody titer to a previous antigen (Rubella vaccine) in 279 children in Missouri aged 9 months to 6 years (mean not reported, 65% had blood Pb <10µg/dL Lutz *et al.* 1999) and there was no difference in tetanus toxoid-specific antibodies between children with very high blood Pb levels (45µg/dL) and high blood Pb levels (23µg/dL) (Reigart and Graber 1976). The few studies that examined serum immunoglobulin levels (other than serum IgE discussed in the previous section) in children or adults with blood Pb levels <10µg/dL report inconsistent results. Serum levels of IgG were correlated (r=0.31; p=0.002) with blood Pb levels (mean <2µg/dL), but IgM was not related to blood Pb in as study of cord blood from 101 newborns in Quebec (Belles-Isles *et al.* 2002). There was no effect of blood Pb level (mean 3µg/dL) on serum IgG, IgM, and IgA in a study of 331 children aged 7-10 in Germany (Karmaus *et al.* 2005). Serum IgG, IgM, and IgA were increased in children with blood Pb≥15µg/dL

relative to children with Pb<5µg/dL in children under the age of 3 at mean blood Pb level of 7µg/dL; immunoglobulin levels were not related to blood Pb in children over 3 years of age or in adults (Sarasua *et al.* 2000). In contrast, serum IgG and IgM were decreased in children in China aged 3-6 with blood Pb >10µg/dL from a sample of 72 children aged 3-6 in china with mean blood Pb of 9.5µg/dL (Sun *et al.* 2003). Blood Pb was not correlated with serum IgA, IgM, or IgG in a study of atopic and nonallergic men in Italy without occupational exposure to Pb (mean Pb=11µg/dL)(n=34 Boscolo *et al.* 1999).

Summary of support for conclusions

Animal data are mixed for an effect of Pb on the antibody response and most studies do not report serum immunoglobulin levels (seeATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). Studies in mice report no effect, suppression, or stimulation of the T dependent antibody response (plaque forming cell [PFC] assay to sheep red blood cell challenge) at blood Pb levels of 25-130µg/dL (Blakley and Archer 1981; Mudzinski *et al.* 1986), while suppression of the PFC response was observed in rats at 29µg/dL (Luster *et al.* 1978). The conclusion of *inadequate* evidence that blood Pb levels <10µg/dL are associated with serum levels of IgG, IgM or IgA or functional antibody response in children or adults is based on the inconsistent results for serum IgG, IgM, and IgA and the general lack of human studies on Pb and the antibody response. The 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead do not make strong conclusions on the antibody response or serum immunoglobulins; however, the NTP's conclusions for *inadequate* evidence for an association between blood Pb levels <10µg/dL and serum IgG, IgM, IgA or the antibody response are consistent with the evidence presented in these documents.

5.3.3 T lymphocytes or T-cells

There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with altered T lymphocytes or T-cell numbers or percentages in the peripheral blood of children or adults (see Appendix B: Immune Effects). The results of studies at lower blood Pb levels (<10µg/dL) are inconsistent; however, there are a number of occupational studies that report decreased absolute numbers or percentages of T-cells or T-cell subsets (particularly CD4+ T-helper cells) and some include a corresponding increase in B-cells at blood Pb above 15 or 30µg/dL. A positive association between blood Pb and naïve T-cells (CD45RA+ or CD45RO-) is reported in several studies at low (<10µg/dL) and high blood Pb levels.

The results of studies in children and adults with blood Pb levels <10µg/dL are inconsistent for a potential relationship between blood Pb and abundance of T-cells. In a study of 70 children aged 3-6 in China, the percentage of T-cells was unchanged, the percentage of CD4 T-cells was decreased and the percentage of CD8 T-cells was increased in children with blood Pb levels above 10µg/dL relative to children <10µg/dL (Li *et al.* 2005; Zhao *et al.* 2004). In contrast, Sarasua *et al.* (2000), reported decreased percentage of T-cells, no change in CD4 or CD8 T-cells and increased percentage of B-cells in children under the age of 3 at mean blood Pb level of 7µg/dL. Lymphocyte populations were not related to blood Pb levels in children over 3 years of age or in adults (Sarasua *et al.* 2000). Four additional studies in children reported no effect of blood Pb on lymphocyte populations at mean blood Pb levels ranging from <2µg/dL to 9µg/dL

in: 318 children <8 years of age in Egypt (Hegazy *et al.* 2011); or 279 children from 9 months to 6 years of age in Missouri (Lutz *et al.* 1999); or 331 children aged 7-10 in Germany (Karmaus *et al.* 2005); and 101 newborns in Quebec (Belles-Isles *et al.* 2002). In adults with blood Pb levels <10µg/dL, the results of studies that examined the relationship between blood Pb and lymphocyte populations are mixed. Blood Pb was positively correlated with CD4 T-cells in atopic and nonallergic men (n=34 Boscolo *et al.* 1999) and positively correlated with CD8 T-cells in nonallergic women, but not atopics in Italy without occupational exposure to Pb (mean Pb 5-11µg/dL)(n=60 Boscolo *et al.* 2000). Blood Pb was also positively correlated with CD4/CD45RO-naive CD4 T-cells in both nonallergic men and women, but not atopics (Boscolo *et al.* 1999; Boscolo *et al.* 2000). Naïve CD4/CD45RA+ CD4 T-cells were also increased in 3-wheel drivers in India (mean Pb=7µg/dL; n=26) relative to referents (mean Pb=5µg/dL; n=59), but CD4+ T-cells were decreased (Mishra *et al.* 2010).

At higher blood Pb levels (i.e., >15µg/dL) there are a number of occupational studies that report altered T or B lymphocyte concentrations. In general, the absolute numbers or percentages of T lymphocytes or T-cell subsets (particularly CD4+ T-helper cells) are decreased and there is a corresponding increase in B-cells. There is no clear and consistent cell population that is related to Pb levels, although a positive association between blood Pb and naïve T-cells is reported in several studies. The number and percent of T-cells and CD4+ T-cells were decreased in firearms instructors in the US (mean Pb 15 [n=36] and 31µg/dL [n=15]) (Fischbein *et al.* 1993). The number of CD4 T-cells were reduced in 25 Pb battery workers in Turkey with very high mean blood Pb levels of75µg/dL (Basaran and Undeger 2000; Undeger *et al.* 1996). In a separate study of Pb battery workers with very high mean blood Pb of 132µg/dL (n=33 in India), Mishra *et al.* (2010) reported that the percentage of CD4 T-cells were decreased and percentages of CD45RA+ (naïve) T-cells were increased. In contrast, Pinkerton *et al*. (1998) reported no effect on CD4 T-cells, that B-cells were positively associated with Pb exposure and that CD4/CD45RA+ naïve CD4 T-cells were negatively correlated with cumulative Pb exposure in a study of US Pb smelter workers (median Pb=39µg/dL; n=145). Several studies have reported that CD4 T-cells were not related to blood Pb, but found either decreases in CD8+ T-cells (Garcia-Leston *et al.* 2011; and decreased CD8 T-cells along with decreased B-cells Kuo *et al.* 2001) or increased percentages of CD8+ T-cells (Sata *et al.* 1998).

The issue of reverse causality is also possible because Pb in whole blood is largely contained in the circulating blood cells, although this is generally attributed to the red blood cells not the white blood cells or leukocytes (lymphocytes are a subpopulation of leukocytes). Choi and Kim (2005) reported that boys with higher blood Pb levels had higher leukocyte counts and leukocyte counts correlated significantly with blood Pb levels (mean 3µg/dL; $r=0.39$; $p<0.05$) in a study of 251 adolescents age 13-15 in South Korea; the authors did not report data for lymphocytes or specific subsets such as T-cells. Higher blood Pb levels in these individuals may cause higher leukocyte counts or higher circulating leukocyte counts may lead to higher measurements of blood Pb if the leukocytes contain substantial amounts of Pb.

Summary of support for conclusions

Animal data support a Pb-associated effect on T-cell maturation, and particularly a shift toward the type 2 helper T-cell (Th-2) phenotype, which is associated with allergy and increased IgE production, versus a type 1 helper T-cell (Th-1) response which is associated with host resistance and delayed type hypersensitivity (DTH) response (reviewed in Dietert and Piepenbrink 2006; U.S. EPA 2006). Some animal data support Pb-associated decreases in T-cell populations (e.g., decreased thymic CD4 and CD8 T-cells with Pb exposure and Listeria infection in BALB/c mice at blood Pb <25µg/dL Dyatlov and Lawrence 2002); however, decreased T-cells populations with Pb exposure are not widely reported in the experimental animal literature. White blood cell differentials with enumeration of lymphocyte subsets (T-cells, B-cells, CD4 and CD8 T-cells) are among the most common immune assays used in human studies due in part to the relative ease of the assay and ability to obtain data from a small blood sample. Values typically have a large degree of variation that is influenced by sex, race, age, and methodological differences in obtaining and processing the samples. It is worth noting that lymphocyte subset analysis is not an evaluation of immune function, although it is an accepted part of a tiered screening approach to the evaluation of potential immunotoxicity of a given chemical (Luster *et al.* 1992). Differential white blood cell counts are not particularly sensitive indicators of immunotoxicity, and statistically significant effects associated with exposure often fall within normal ranges for the population. Pb-associated changes in T-cell counts or percentages at lower exposure levels are relatively small and may be without a functional impact on the immune response of individuals. However, on a population level, a small change in cell numbers or percentages may be adverse. A good example is the demonstration that increased mortality risk is associated with small decreases in CD4 and total white blood cell counts in a study of people 85 and older (Izaks *et al.* 2003).

Although there is no clear or consistent cell population that is related to Pb levels, changes in T-cell populations are reported in a wide range of human studies. Decreased T-cells or CD4 T-cells (particularly at blood Pb >15µg/dL) and increased naïve T-cells may be a biological signal of Pb exposure in humans. The NTP's conclusion that there is *inadequate* evidence that blood Pb levels <10µg/dL are associated with altered T-cell abundance in the peripheral blood of children or adults is based on the lack of a clear pattern of results in studies with lower blood Pb levels. Changes in T-cell populations do not equate to a functional immune outcome, although they may be relevant to changes in the DTH response. Although there is a large body of data from animals that demonstrates clear and consistent Pb-associated suppression of the DTH response in mice, rats, goats, and chickens (see discussion of the animal data on DTH below and Dietert and Piepenbrink 2006 for review; U.S. EPA 2006), no studies of Pb and the DTH response were located in humans. The EPA 2006 AQCD for Lead (U.S. EPA 2006) and 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) both include changes in T-cell subpopulations as characteristic immune effects identified with Pb exposure at higher levels (e.g., 30-70µg/dL in ATSDR).

5.3.4 Monocyte/Macrophages

There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with changes in macrophage function. The human data on macrophage function are limited to a single study of

65 children aged 6-11 in Mexico living near a Pb smelter (mean blood Pb levels of 21 and 30µg/dL) compared to referents with blood Pb levels of 7µg/dL (Pineda-Zavaleta *et al.* 2004). The study investigated nitric oxide (NO) and increased superoxide (O_2^-) production by macrophages. At appropriate levels, both NO and O_2^- are involved in destruction of bacteria by macrophages and other cells. Decreased macrophage nitric oxide (NO) and increased superoxide (O_2^-) production following indirect (PHA) stimulation through lymphocytes as well as direct (IFNγ-LPS) stimulation of the macrophages were observed in cell cultures from the Pb exposed boys, but not the girls. Animal data provide strong support for decreased NO and increased O_2^- or reactive oxygen intermediate production by macrophages following *in vivo* or *in vitro* exposure to Pb, but generally lack blood Pb data (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). Production of the proinflammatory cytokine TNF-α by macrophages is also associated with Pb exposure and the animal data suggest that production of TNF-α by macrophages is linked to increased sensitivity to bacterial derived endotoxin (see U.S. EPA 2006 for recent reviews of the animal data). *In vitro* studies of human peripheral mononuclear cells (including macrophages) demonstrated increased production of TNF-α with Pb exposure (Villanueva *et al.* 2000) in the presence of LPS plus Pb (Guo *et al.* 1996). However, low levels of Pb were associated with suppression of TNF-α release in human peripheral mononuclear cells in the presence of heat-killed *Salmonella enteritidis* (Hemdan *et al.* 2005). The 2006 EPA AQCD for Lead identifies a stimulation of a hyperinflammatory state in macrophages as one of the principal immune effects of Pb; however, it notes that there is a general lack of human epidemiological data in this area.

5.3.5 Neutrophils

There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with changes in neutrophil function. There are limited human data on the potential association between Pb exposure and neutrophils, and the studies are restricted to occupationally exposed individuals with mean blood Pb levels that are >30µg/dL (see Appendix B: Immune Effects). Several studies report that neutrophil chemotaxis may be reduced in Pb workers at high blood Pb levels (Governa *et al.* 1988; Queiroz *et al.* 1993) or following in vitro exposure to Pb (Governa *et al.* 1987). There is also evidence that lytic activity of Candida may be reduced (Queiroz *et al.* 1994) but phagocytic activity is relatively unaffected in Pb workers (Guillard and Lauwerys 1989; Queiroz *et al.* 1994). There is little animal data on neutrophil function, and in humans there is a complete lack of data with lower blood Pb levels. Additional studies of neutrophil function are required to clarify the potential relationship to blood Pb in adults and children. The 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead identifies neutrophil chemotaxis as a consistent finding with Pb exposure; however the studies do not include blood Pb levels <10µg/dL.

5.4 Susceptible Populations or Life stages

Segments of the population that are may be more susceptible to health effects of Pb are discussed more extensively in **Section 3.0 Exposure**. There is a significant body of literature supporting developmental period as a susceptible window for immunotoxicity, with immune effects of developmental toxicant exposure occurring at lower doses and adverse effects may

be more persistent than similar exposure of adults (Dietert 2008; Luebke *et al.* 2006). As described above, the database of immune studies of Pb in humans provides *limited* evidence that blood Pb levels <10µg/dl are associated with increased hypersensitivity responses and allergic sensitization diagnosed by skin prick testing in children; however, there is *inadequate* evidence to assess these endpoints in adults. Children may represent a sensitive life stage for the effects of Pb on IgE and IgE-related effects, but there is not enough data on these endpoints in adults with low blood Pb levels to make this determination.

There are some established age-related differences in immunity including a general decline in IgE and allergic symptoms with increasing age that is more pronounced in the elderly (Mediaty and Neuber 2005). However, there is some evidence that the apparent age-related decrease in hypersensitivity may be associated with more than age-related decline, and the difference may reflect an increase in IgE and sensitization rates in recent cohorts (Jarvis *et al.* 2005). In either case, childhood and prenatal exposure periods are potentially susceptible life stages due to the elevated background level of IgE and IgE-mediated hypersensitivity. In addition, young children have higher levels of Pb exposure related to early hand-to-mouth behaviors. Therefore, children may experience higher levels of Pb during a life stage that is already characterized by elevated IgE.

There are also gender-related differences in immune function, with many studies reporting higher total IgE levels in boys and men than in girls or (e.g., see Raby *et al.* 2007). A recent meta-analysis of over 550 studies reported that boys make up 64% of children age 18 or younger with allergies, but that women make up 65% of adults above the age of 18 with allergies (Kelly and Gangur 2009). The Pb epidemiological data include two studies that report significant modification by gender of the effects of Pb on IgE. Sun *et al.* (2003) reported that increased IgE was statistically significantly correlated with blood Pb in girls in China with blood Pb≥10µg/dL aged 3-6, but that the effect was not significant in boys. Similarly, Pizent *et al.* (2008) reported that serum IgE was correlated to blood Pb levels in female office workers in Croatia, but not in the men. These data suggest that females may be more susceptible to the effects of Pb on IgE and allergy. Alternatively, the higher basal IgE levels in males may make it more difficult to detect the effects of Pb.

The risk factors for hypersensitivity and allergies include age and sex discussed above, but evidence suggests that heredity is by far the most important (De Swert 1999). This may manifest as a difference by race; for example, Joseph *et al.* (2005) reported that African American children were at statistically significantly greater risk of asthma compared to Caucasians, regardless of blood Pb level. The general or background allergic status of mothers or of children may be relevant to the effects of Pb on IgE and hypersensitivity. Annesi-Maesano reported that the association between infant hair Pb levels and cord blood IgE were affected by the allergic status of the mothers. In analyses dividing mothers by history of IgE-mediated allergic status (either allergic as indicated by asthma, allergic rhinitis, or eczema) or non-allergic, the study reported that infant hair Pb was statistically significantly associated with increased IgE in children born to non-allergic mothers ($r=0.21$; $p<0.01$), but the relationship was not significant for children born to allergic mothers ($r=0.12$; $p>0.05$). The authors suggest that

family history of allergy (in other words, atopy) may overshadow the effect of Pb on IgE in children. It is unclear from this data whether this reflects genetic factors predisposing the children to allergy (e.g., Hunninghake *et al.* 2011; Hunninghake *et al.* 2008; Raby *et al.* 2007) or whether prenatal exposure to cytokines, histamine or other factors are important. In the one prospective study available, Jedrychowski *et al.* (2011) controlled for maternal allergic history (or atopy), in the analysis that demonstrated a statistically significant positive association between cord blood Pb and atopic status as indicated by a positive skin prick test to at least one common allergen in the children at 5 years of age. Furthermore, adjustment for maternal atopy, as well as child's gender, parity, maternal age, maternal education, and environmental tobacco, had very little effect on the relative risk (RR = 2.20(95% CI:1.17,4.16) before adjustment and R=2.28(95%CI:1.12,4.62) after). The association with maternal blood Pb also had a relatively small effect, but it did move it from statistically significant to borderline (RR = 1.81(95% CI:1.10,3.00) before adjustment and R=1.72(95%CI:0.98,3.00) after) (Jedrychowski *et al.* 2011).

5.5 Pb Exposure Measurements

The following brief discussion outlines several Pb exposure issues that are directly relevant to immune effects of Pb. An expanded discussion is included in a separate Section of this document (see **Section 3.0 Exposure**). No studies of immune effects in humans were located that used an exposure metric other than blood Pb and hair Pb. However, it is important to note that blood Pb is only one exposure metric and it reflects a portion of the Pb that is present in a given subject. The half-life of Pb in blood is approximately 35 days and therefore blood Pb is considered a good indicator of recent exposure. The majority of Pb, approximately 90% in adults, 80% in adolescents, and 66% in children under 5 years of age is stored in bones and Pb from past environmental exposure is released into the blood contributing to a chronic endogenous source of exposure (Leggett 1993).

The higher levels of Pb in bone may be particularly relevant for cells of the immune system and immune function. All of the white blood cells or leukocytes that develop postnatally are derived from progenitor cells in the bone marrow in a process termed hematopoiesis. Elevated Pb concentrations in bone are therefore of direct concern for the development of immune cells and future studies should more closely consider the potential relationship between bone Pb levels and immune effects. Mechanistic studies in animals support the importance of Pb exposure on the development and differentiation of leukocytes. For example, Gao *et al.* (2007) reported that Pb modified bone marrow-derived dendritic cells to promote Th-2 phenotype and immune responses associated with allergy and increased IgE production. The location of Pb within blood is also of particular relevance to immune function. The Pb in blood is principally contained in cells, primarily in red blood cells, with less than 1% in serum. As discussed in **Section 5.3.3.T lymphocytes or T-cells** above, Choi and Kim (2005) reported that blood Pb concentrations (mean 3µg/dL) were 2 fold higher in boys with higher leucocyte counts; and, leukocyte counts correlated significantly with blood Pb levels ($r=0.39$; $p<0.05$) in a study of 251 adolescents age 13-15 in South Korea. The issue of reverse causality is suggested and higher blood Pb levels in these individuals may cause higher leukocyte counts or higher circulating

leukocyte counts may result in elevated blood Pb concentrations if the leukocytes contain substantial amounts of Pb in the boys in this study.

5.6 Delayed type hypersensitivity (DTH) and Pb-related Immune Effects in Animal Studies

There is a large body of laboratory animal data on immune effects of Pb. The effects in animal models support the human data as discussed above for increased IgE with evidence to support a Th-2-related mechanism and proinflammatory shift in macrophage function. However, the major effect on immune function associated with Pb exposure appears to be suppression of the delayed type hypersensitivity (DTH) response. Animal data provide strong and consistent support for Pb-associated suppression of DTH response (seeATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The DTH response depends on priming and expansion of antigen specific T-cells that are Th-1 dependent and therefore the Pb-associated suppression of Th-1 responses is consistent with the Pb-associated suppression of DTH. The DTH response is a functional immune endpoint that is a widely accepted indicator of cell-mediated function (Luster *et al.* 1992; U.S. EPA 1998). Although DTH response can be evaluated as a measure of immune response in humans (e.g., Vukmanovic-Stejic *et al.* 2006), no studies were located that evaluate the relationship between blood Pb and DTH in humans. Studies on the DTH response in humans with low blood Pb levels are recommended because of the lack of data in humans and the clear and consistent Pb-related suppression of the DTH response in mice, rats, goats, and chickens.

Exposure to Pb is associated with suppression of the DTH response in mice, rats, goats and chickens and following both acute and subchronic exposure up to 16 weeks in length. Although blood Pb levels are not available in all studies, decreased DTH response has been reported at blood Pb levels from 29 to 87µg/dL. Wistar rats that were the offspring of dams exposed from premating to weaning, and continued to receive 25 or 50 ppm Pb acetate in drinking water until tested, had suppressed DTH at blood Pb level of 29 and 52µg/dL (Faith *et al.* 1979). Adult BALB/c mice exposed to 32, 128, 512, or 2048 ppm Pb acetate in drinking water for 3 weeks had blood Pb levels of 9, 49, 87, and 169µg/dL and the blood Pb level correlated with suppressed DTH response (McCabe *et al.* 1999). Although McCabe *et al.* (1999) report that the DTH response in the mice with 87µg/dL blood Pb level was statistically suppressed, it is not clear from the paper if blood Pb level of 9µg/dL (a more environmentally relevant level) was associated with suppressed DTH response. In a recovery study with maternal exposure of Fisher 344 rats to 250 ppm Pb acetate, maternal blood Pb levels as high as 66µg/dL; however, there was no effect of Pb exposure in the dams 8 weeks after parturition and stopping the Pb exposure (Chen *et al.* 2004). In contrast, the offspring with blood Pb levels between 6 and 8µg/dL measured 4 weeks after the dams were last exposed had significantly suppressed DTH response. The developmental nature of this study and the early removal of Pb exposure to the dams suggest that the DTH effect of Pb has a clear developmental component. There are no experimental animal data that report a blood Pb level associated with a no effect level, and therefore the lower blood Pb range associated with effects is unknown, even in laboratory animals.

5.7 Conclusions

The NTP concludes that there is *limited* evidence that blood Pb levels <10µg/dL are associated with adverse immune effects in children and there is *inadequate* evidence in adults (see **Table 5.4** for complete list of immune effects conclusions). In children, there is *limited* evidence that blood Pb levels<10µg/dL are associated with increased hypersensitivity responses and allergic sensitization diagnosed by skin prick testing to common allergens. There is also *sufficient* evidence that blood Pb levels of 10µg/dL and below are associated with elevated serum IgE levels. The data supporting Pb-related increases in IgE, together with a prospective study on allergic sensitization, provide *limited* evidence that blood Pb <10µg/dL is associated with increased hypersensitivity responses in children. In some studies blood Pb levels at and below 2µg/dL are associated with increased serum IgE (e.g., Hon *et al.* 2010; Karmaus *et al.* 2005) or increased sensitization to common allergens indicated by positive skin prick test (e.g., Jedrychowski *et al.* 2011). There is *inadequate* evidence of an association between blood Pb and eczema or asthma in children and there is *inadequate* evidence in adults to address the potential association between blood Pb <10µg/dL and IgE, allergy, eczema, or asthma. There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with observational data such as altered lymphocyte counts or serum levels of IgG, IgM or IgA in the peripheral blood of children or adults because of a general lack of studies at the lower dose and inconsistency in available data. There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with changes in immune function other than hypersensitivity because there are few studies of Pb and immune function in humans, particularly at lower blood Pb levels. Very few studies examine markers of exposure other than blood Pb levels, and therefore it is unknown if blood or bone Pb levels would be a better indicator of immune effects.

Table 5.4: NTP conclusions on Immune effects of low level Pb				
Health Effect	**Population Or Exposure window**	**NTP Conclusion**	**Blood Pb Evidence**	**Bone Pb Evidence**
Increased serum IgE	Prenatal	*Inadequate*	Unclear	Hair Pb data
	Children	*Sufficient*	Yes, <10µg/dL	No data
	Adults	*Inadequate*	Unclear	No data
Increased Hypersensitivity and Allergy (e.g., positive skin prick test)	Prenatal	*Limited*	Maternal and cord<10µg/dL	No data
	Children	*Limited*	Yes, <10µg/dL	No data
	Adults	*Inadequate*	Unclear	No data
Asthma, Eczema, etc.	Prenatal	*Inadequate*	Unclear	No data
	Children	*Inadequate*	Unclear	No data
	Adults	*Inadequate*	Unclear	No data
Altered serum IgG, IgM	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	Unclear	No data
	Adults	*Inadequate*	Unclear	No data
Altered antibody response	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	No data	No data
	Adults	*Inadequate*	No data	No data
Immunophenotyping (e.g., T-cells, B-cells)	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	Unclear	No data
	Adults	*Inadequate*	Unclear >15µg/dL data suggest changes in T-cells or T-cell subpopulations	No data
Monocyte/Macrophage function	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	Unclear (one study)	No data
	Adults	*Inadequate*	No data	No data
Neutrophil function	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	No data	No data
	Adults	*Inadequate*	Unclear >30µg/dL data suggest changes in chemotaxis and lytic activity	No data
Delayed type Hypersensitivity (DTH) response	Prenatal	*Inadequate*	No data	No data
	Children	*Inadequate*	No data	No data
	Adults	*Inadequate*	No data	No data

6.0 CARDIOVASCULAR EFFECTS

6.1 Conclusions

NTP concludes that there is *sufficient* evidence that blood Pb levels <10 µg/dL in adults are associated with adverse effects on cardiovascular function.

There is *sufficient* evidence of a bone Pb-related increase in blood pressure (BP) and the risk of hypertension. Two prospective studies and five cross-sectional studies found a statistically significant association between bone Pb and increased BP or hypertension. These studies were in populations with blood Pb levels below 10µg/dL, and the majority of them had mean levels below 5µg/dL. Blood Pb was less consistently associated with BP and hypertension in adults. Studies of populations with mean blood Pb levels <5µg/dL (often more recent studies) have found significant associations between concurrent blood Pb and higher BP. The NTP recognizes that an individual with blood levels <10µg/dL during adulthood may have had higher blood Pb levels earlier in life, and the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb.

There is *sufficient* evidence of increased risk of cardiovascular-related mortality associated with blood Pb levels <10µg/dL in two prospective studies and bone Pb in one prospective study (mean blood Pb = 5.6µg/dL). There is *sufficient* evidence that blood Pb in pregnant women is associated with an increased risk of gestational hypertension with blood Pb levels <10µg/dL. One prospective study and five cross-sectional studies supported an association, all with mean blood Pb levels <10µg/dL. There is *limited* evidence for Pb effects on other cardiovascular outcomes including heart rate variability, ECG abnormalities, and incident cardiovascular disease including cerebrovascular disease and peripheral arterial disease; due to few replicated studies of blood PB effects.

There is *inadequate* evidence to assess whether children present a sensitive life stage for cardiovascular effects of Pb. No prospective studies have followed children with early life Pb measures with determination of cardiovascular health after childhood, and the few studies of blood Pb and BP during childhood were inconsistent. During menopause and with osteoporosis, bone Pb stores are mobilized, increasing circulating Pb levels and putting women at greater risk of Pb-related cardiovascular effects. There is *inadequate* evidence to assess cardiovascular effects of Pb in menopausal women. One cross-sectional study (mean blood Pb) found a stronger statistically significant association between blood Pb and hypertension in post-menopausal women (Nash *et al.* 2003), but two smaller studies found no association (Al-Saleh *et al.* 2005; Pizent *et al.* 2001).

Chronic Pb exposure appears to be more critical than current Pb exposure as indicated by more consistent associations of bone Pb with chronic cardiovascular effects such as hypertension and mortality from cardiovascular causes, as compared to studies of blood Pb. The data are inadequate to evaluate prenatal or childhood Pb exposure with health outcomes at later stages of development or in adulthood.

6.2 How Conclusions Were Reached

Conclusions in the NTP evaluation of Pb-related cardiovascular effects in humans associated with low-level Pb were derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10µg/dL. The NTP's conclusions are based on the evidence from human studies with blood Pb levels of ≤10µg/dL with data reflecting exposure levels up to 15µg/dL. This section of the evaluation focuses primarilyon the human data for cardiovascular effects of Pb because there is a relatively large database of human studies for these endpoints. Major endpoints considered as potential indicators of effects of Pb on cardiovascular functions are listed and briefly described in **Section 6.2.1.** This document is not a review of the cardiovascular system or toxicity and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP conclusions are discussed in detail in **Section 6.3 Evidence for Pb-related Effects on Cardiovascular Outcomes**. The discussion of each cardiovascular effect begins with a statement of the NTP conclusion whether the specific effect is associated with a blood Pb level <10µg/dL or <5µg/dL and the age group in which it is identified (childhood or adulthood). The majority of studies are prospective or cross-sectional within a life stage (childhood or adulthood exposure and outcome) unless otherwise indicated. The discussion also highlights the extent to which experimental animal data support the association between Pb exposure and cardiovascular effects. Although the information necessary to support the NTP conclusions is presented in **Section 6.3**, the complete dataset of human studies with data on cardiovascular endpoints from Pb exposed populations is included in the Cardiovascular Appendix and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 6.2.2** below.

6.2.1 Principal Measures of Cardiovascular Effects

Table 6.1 lists a number of key cardiovascular endpoints commonly evaluated in epidemiological studies (as defined by the American Heart Association Cardiac Glossary (http://www.heart.org/HEARTORG/Conditions/HeartAttack/HeartAttackToolsResources/Cardiac-Glossary_UCM_303945_Article.jsp or studies of pulse pressure and heart rate variability cited below). Blood pressure (BP) is the most widely measured cardiovascular effect in studies of Pb exposure and is evaluated as a continuous measure (in mmHg) or as a dichotomized measure (hypertension). High BP increases the risk of myocardial infarction and stroke, and blood pressure control is one of the primary strategies to prevent the development of cardiovascular disease with evidence of efficacy in patients without prior heart disease (Law *et al.* 2009). Pulse pressure is the difference between systolic and diastolic blood pressure (SBP and DBP) and increases reflect arterial stiffness, a critical cardiovascular risk factor (Lakatta and Levy 2003). Heart rate variability is an indicator of cardiac autonomic function, with decreased variability being associated with risk of heart disease and mortality, and with clinical relevance in relation to Pb exposure (Park *et al.* 2006).

Electrocardiographic (ECG) conduction abnormalities have also been investigated in relation to Pb exposure. Major clinical cardiovascular endpoints that have been associated

Table 6.1: Major Pb-related cardiovascular outcomes/effects

Cardiovascular Effect	Description
Blood Pressure (BP)	The force exerted by the heart against the walls of the arteries measured in millimeters of mercury (mmHg), with a maximum during the pumping phase of the heartbeat (systolic, SBP) and a minimum when the heart muscle relaxes between beats (diastolic, DBP)
Hypertension	Medical term for high blood pressure (currently, systolic ≥140 or diastolic ≥90) compared to an optimal BP of less than 120/80 mmHg. BPs of 120-139/80-89 mmHg are prehypertension
Pulse Pressure	The difference between SBP and DBP; a marker of arterial stiffness
Heart Rate Variability	Changes in the interval between heart beats; decreased variability is a marker of abnormal autonomic nervous system functioning
Electrocardiographic (ECG) Conduction Abnormalities	Changes in the typical pattern of electrical activity of the heart including the P wave (atria), QRS wave (ventricles) and T wave (return to resting state)
Peripheral Artery Disease	Narrowing of arteries carrying blood to the arms and legs, caused by atherosclerosis
Coronary Heart Disease	Narrowing of the arteries that supply blood and oxygen to the heart muscle caused by atherosclerosis and can result in a myocardial infarction (also called Ischemic Heart Disease)
Myocardial Infarction	Medical term for a heart attack, damage to heart muscle resulting from a blocked blood supply
Stroke	Death or injury to brain cells when a blood clot blocks an artery in or leading to the brain (Ischemic) or when a blood vessel ruptures (Hemorrhagic)
Cardiovascular Mortality	Death attributed to heart or circulatory causes

American Heart Association Cardiac Glossary (http://www.heart.org/HEARTORG/Conditions/HeartAttack/HeartAttackToolsResources/Cardiac-Glossary_UCM_303945_Article.jsp)

with Pb exposure include peripheral artery disease, coronary heart disease (ischemic heart disease) and stroke (cerebrovascular disease). Cardiovascular disease mortality has also been related to lead exposure.

The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 6.3 Evidence for Pb-related Effects on Cardiovascular Outcomes** below.

6.2.2 Principal conclusions from the 2006 EPA and 2007 ATSDR Pb documents

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both concluded that epidemiological studies support a negative relationship between Pb exposure and cardiovascular health including increased SBP and DBP, higher incidence of hypertension, and increased incidence of cardiovascular disease and cardiovascular-related mortality (see **Table 6.2** for principal conclusions and original documents for complete conclusions). The association between elevated blood Pb and increased BP (systolic and diastolic) is supported by a large body of literature including cross-sectional (e.g., NHANES), prospective cohort (e.g., Boston Normative Aging study), and occupational studies as well as several meta-analyses (Nawrot *et al.* 2002; Schwartz 1995; Staessen *et al.* 1994). EPA (U.S. EPA 2006) and ATSDR (ATSDR 2007) both stated that the data support an increase of approximately 1.0 mmHg in systolic and 0.6 mmHg in diastolic BP for every doubling of the blood Pb level. Both agencies concluded that cumulative past Pb exposure, indicated by bone Pb, may have a stronger association with increases in blood pressure than current exposure as indicated by blood Pb level. Every 10µg/g increase in bone Pb was associated with an odds ratio for hypertension of 1.28 to 1.86 over a bone Pb range of <1.0µg/g to 96µg/g (U.S. EPA 2006). ATSDR also highlighted the potential mechanistic link between cardiovascular and renal effects of Pb.

Table 6.2: Main conclusion for cardiovascular effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"Epidemiologic studies support the relationship between increased lead exposure and increased deleterious cardiovascular outcomes, including increased blood pressure and increased incidence of hypertension. … The evidence for an association of Pb with cardiovascular morbidity and mortality is limited but supportive." (EPA, 2006 pg 6-271)
"Population studies suggest that there is a significant association between bone-lead levels and elevated blood pressure. Blood lead levels (PbBs) also have been associated with small elevations in blood pressure." (ATSDR, 2007 pg 21)

EPA: United States Environmental Protection Agency
AQCD: Air Quality Criteria Document
ATSDR: Agency for Toxic Substances and Disease Registry

The NTP accepted the conclusions from the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) on Pb-related cardiovascular effects. The focus of EPA's document did not clearly discriminate effects below and above 10µg/dL. Most of the studies of the quantitative relationship between blood Pb and systolic BP report mean blood Pb levels below 10µg/dL (e.g. table 6-2 in U.S. EPA 2006). However, the conclusion on the increase in BP associated with a doubling of blood Pb, did not specifically state if this doubling occurred at blood Pb levels < 10µg/dL. Some studies considered by EPA, particularly

those conducted before 1990 or in occupational settings, had more than 90% of their subjects with blood Pb >10µg/dL (Gartside 1988; Kromhout *et al.* 1985; Lockett and Arbuckle 1987). Such studies were not considered for the NTP conclusions on Pb effects below 10µg/dL.

6.3 Evidence for Pb-related Effects on Cardiovascular Outcomes

6.3.1 Blood Pressure (BP) and Hypertension

There is *sufficient* evidence that blood Pb levels <10µg/dL are associated with increases in blood pressure and risk of hypertension (see the Blood Pressure (BP) and Hypertension Section of the Cardiovascular Appendix). BP is the most widely studied cardiovascular measure in studies of Pb, as it is routinely and easily measured. A positive association between Pb level and blood pressure is most consistent in studies of bone Pb (see **Table 6.3**). Women are at particular risk as there is *sufficient* evidence that blood Pb <10µg/dL increases the risk of hypertension during pregnancy. Adults with concurrent blood Pb levels <10µg/dL may have had higher Pb levels in the past and the role of current blood Pb cannot be separated from an effect of early-life Pb exposure.

There is *inadequate* evidence for Pb effects on BP or hypertension in children. The size of the increase in BP that is detected is relatively small (1-2 mmHg). It is well established, however, that small increases in BP levels at the population level can have a substantial public health impact increasing the risk of hypertension and incident cardiovascular disease (Whelton *et al.* 2002).

Despite some inconsistency across human studies, meta-analyses conclude that Pb exposure is associated with increased BP levels (Navas-Acien *et al.* 2008; Nawrot *et al.* 2002). Meta-analyses can account for the relative contributions of each study and multiple publications on overlapping datasets. Several recent meta-analyses support a small increase in BP from both blood (Nawrot *et al.* 2002) and bone Pb (Navas-Acien *et al.* 2008). Small, but significantly increased risks of hypertension were found with tibia Pb (for a 10µg/g increase OR=1.04, 95% CI 1.01-1.07), and nonsignificant increases for patella Pb (for a 10µg/g increase OR=1.04 95% CI 0.96-1.12) and blood Pb (for a 5µg/dL increase OR=1.02, 95% CI 0.93-1.13) (Navas-Acien *et al.* 2008). Neither meta-analysis focused on low-level Pb exposure; for example, Navas-Acien et al. (2008) included two studies with mean blood Pb levels over 30µg/dL, while the other eight were below 10µg/dL.

Bone Pb: Long term exposure to Pb is often reflected in bone Pb levels, and several studies reported a significant association of bone Pb with BP, but not blood Pb (Cheng *et al.* 2001; Gerr *et al.* 2002; Rothenberg *et al.* 2002). However, bone Pb must be measured during specialized clinic visits and has not been as widely studied. **Table 6.3** summarizes the bone Pb literature for BP and hypertension listed by study type and decreasing size, grouped together for overlapping or shared study populations. Most cross-sectional studies found an association with bone Pb and hypertension in the general population (Elmarsafawy *et al.* 2006; Hu *et al.* 1996; Martin *et al.* 2006; Rothenberg *et al.* 2002). One cross-sectional study did not find bone

Table 6.3: Studies of the association between bone Pb and blood pressure and hypertension used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
Supporting	496 former Pb workers, USA	Prospective	In men with past occupational Pb exposure (mean blood 4.6µg/dL), blood and tibia Pb were associated with annual increases in SBP, but not DBP, over 3 years of follow-up.	Glenn (2003)
Normative Aging Study, USA				
Supporting	474 men	Prospective	Bone Pb was correlated with increased SBP at baseline and an increased risk of hypertension 3 to 6 years later, while blood Pb was not associated.	Cheng (2001)
	619 men	Cross-sectional	A positive association between pulse pressure and bone Pb in a population of older men is modified by genetic variations in the *HFE* gene.	Zhang (2010)
	593 men	Cross-sectional	In a population of older men, tibia Pb was positively association with pulse pressure, but not blood Pb which was positively correlated with DBP.	Perlstein (2007)
	590 men	Cross-sectional	Bone and blood Pb levels were higher in hypertensives (mean blood <7µg/dL), and tibia Pb independently increased the risk of hypertension.	Hu (1996)
	471 men	Cross-sectional	The relationship between blood Pb, tibia Pb, and patella Pb and hypertension or BP may be modified by dietary calcium intake in a population of men with low Pb levels.	Elmarsafawy (2006)
Not Supporting	750 older men (513 with hypertension	Case-control	Bone Pb in the patella and tibia were not associated with risk of hypertension in these older men with low blood Pb levels (mean 6.3g/dL).	Peters (2007)
Supporting	667 third trimester or postpartum women, USA	Cross-sectional	Calcaneus bone Pb measured postpartum was associated with increased BP and hypertension during the third trimester (mean blood Pb 2.3µg/dL postpartum).	Rothenberg (2002)
Supporting	964 adults, Baltimore Memory Study, USA	Cross-sectional	In these older adults blood Pb (mean 3.5µg/dL) was associated with BP, while bone Pb was associated with risk of hypertension.	Martin (2006)
Supporting	543 former Pb workers, USA	Cross-sectional	In men with prior occupational exposure, blood Pb was associated with increased BP and an increased risk of hypertension, but not tibia or DMSA chelatable Pb.	Schwartz (2000)
Supporting	508 young adults, half lived near Pb as children, USA	Cross-sectional	In young adults, some with childhood Pb exposure, SBP and DBP was significantly increased in those with the highest bone Pb levels (>10µg/g, blood Pb 3.15µg/dL).	Gerr (2002)
Supporting	284 women (89 with hypertension), Nurses' Health Study	Case-control	Patella Pb was associated with an increased risk of hypertension in these middle aged women without occupational exposures (mean 3µg/dL), but tibia and blood Pb were not significantly associated.	Korrick (1999)

Epidemiological studies of bone Pb exposure and blood pressure and hypertension listed by study type and decreasing size, grouped together for overlapping or shared study population.
BP, SBP, DBP: Blood Pressure, Systolic Blood Pressure, Diastolic Blood Pressure
DMSA: dimercaptosuccinic acid, used in the treatment of Pb poisoning

Pb to significantly increase the risk of hypertension, while blood Pb was significantly associated (Schwartz and Stewart 2000).

Blood Pb: Studies of blood Pb and BP do not consistently support an association as compared to studies of bone Pb and BP (see Blood Pressure (BP) and Hypertension Section of the Cardiovascular Appendix). One prospective study supports a modest increase in SBP with both blood and bone Pb (Glenn *et al.* 2003) while three publications from two prospective studies failed to show an association with SBP or DBP (Grandjean *et al.* 1989; Moller and Kristensen 1992; Staessen *et al.* 1996). The prospective studies that did not find a significant association were in populations with higher mean blood Pb levels (between 10 and 15µg/dL at baseline in Møller (1992) and Staessen (1996)) than the supportive study (4.6µg/dL in Glenn (2003)). The Glostrup Population Study, which did not support an association, was also larger than the supportive cohort (1052 subjects in Møller (1992) vs. 496 in Glenn (2003)). Thus the studies which do not support an association between blood Pb levels and increased BP are not necessarily underpowered or less exposed than the supportive studies. Twenty-nine publications of cross-sectional analyses with mean blood Pb levels <15µg/dL support a small increase in SBP or DBP, while 17 did not support a relationship (some studies had multiple publications, so not all results are independent, see Blood Pressure (BP) and Hypertension Section of the Cardiovascular Appendix for a complete list of studies considered). Analysis of NHANES 1999-2006 restricted to subjects with blood Pb <10µg/dL (n=16,222) found a significant association with increased SBP and DBP (Scinicariello *et al.* 2011).

Three prospective studies failed to find an association between blood Pb and hypertension (Cheng *et al.* 2001; Grandjean *et al.* 1989; Staessen *et al.* 1996), although one of them did find an association with bone Pb (Cheng *et al.* 2001). Blood Pb was associated with increased prevalence of hypertension in ten cross-sectional studies , but there was no association in one study (Kim *et al.* 2008). Black men had a significantly increased risk of hypertension (adjusted prevalence odds ratio 2.69 (95% CI 1.08 – 6.72) for 90^{th} (≥3.50 µg/dL) vs. 10^{th} (≤0.70 µg/dL) percentile) when restricted to 1767 subjects with blood Pb <10µg/dL from NHANES 1999-2006 (Scinicariello *et al.* 2011). Case-control studies of hypertension were also inconsistent: supporting blood Pb (Bakhtiarian *et al.* 2006), not supporting blood Pb while supporting bone lead (Korrick *et al.* 1999), and not supporting either blood (Al-Saleh *et al.* 2005) or bone Pb (Peters *et al.* 2007).

Pulse pressure: Higher pulse pressure (the difference between SBP and DBP) is a marker of arterial stiffness, and there was no association with blood Pb in the Normative Aging, but tibia Pb above the median was associated with an increase of 4 mmHg in pulse pressure (Perlstein *et al.* 2007; Zhang *et al.* 2010). In Mexican-American male NHANES subjects with blood Pb <10µg/dL (n=1925), there was a significant 1.4mmHg increase in pulse pressure per unit increase in the natural log of blood Pb (Scinicariello *et al.* 2011).

Differential Impacts: Blood Pb and bone Pb may reflect variable cardiovascular effects of Pb with acute effects on transient measures, such as BP; and chronic effects on clinical disease, such as hypertension - the more permanent state of elevated BP (Navas-Acien *et al.* 2008).

Martin *et al.* (2006) proposed this hypothesis when they reported a significant association between blood Pb and BP as well as between bone Pb and hypertension. A prediction model for bone Pb based on the Normative Aging Study was developed by Park *et al.* (2009b). When it was applied to data from NHANES III, they found relatively more significant associations between estimated bone Pb and hypertension (Park *et al.* 2009b). However, it should be noted that the model building NAS population only included older men and factors such as age, menopause, and past pregnancies are associated with the mobilization of bone Pb (Symanski and Hertz-Picciotto 1995).

Modifiers: Blood pressure itself is a transient measure influenced by important cofactors that may modify an association with Pb (see **Section 6.4 Susceptible Populations and Modifiers of Pb Exposure**). The increase in BP that is supported by the data is 1-2mmHg per doubling of blood Pb, and on a population basis the impact is likely to be larger in susceptible populations such as pregnant women or individuals with a particular metabolic gene variant.

Pregnancy puts women at greater risk of hypertension, which can contribute to preeclampsia and other complications. The mobilization of bone Pb during pregnancy (Gulson *et al.* 1997; Rothenberg *et al.* 2000) (see further discussion in **Section 3.4 Modifiers of Pb Exposure**) may contribute to the stronger association of Pb exposure with hypertension and the greater magnitude of the change in BP during pregnancy (see **Table 6.4**). Blood Pb levels were associated with hypertension during pregnancy in a prospective study (Sowers *et al.* 2002), cross-sectional studies (Rabinowitz *et al.* 1987; Yazbeck *et al.* 2009), and a case-control study (Vigeh *et al.* 2004). One study did not find an association between cord blood Pb and gestational hypertension, but did find a significant association with maternal SBP and DBP prior to delivery (Wells *et al.* 2011). All of these studies had mean blood Pb levels <10µg/dL, and two of the supportive studies were had mean Pb levels <2µg/dL (Sowers *et al.* 2002; Yazbeck *et al.* 2009). Additional studies in pregnant women support a positive association between blood Pb and BP (Magri *et al.* 2003; Rothenberg *et al.* 1999) and bone Pb and BP (Rothenberg *et al.* 2002).

Women experiencing menopause may be at an increased risk due to mobilization of bone Pb stores (Silbergeld *et al.* 1988; Symanski and Hertz-Picciotto 1995). In NHANES III women aged 40-59 untreated for hypertension, the association between blood Pb and hypertension was stronger in post-menopausal women with statistically significant odds ratios for systolic hypertension of 3.0 (95% CI 1.3-6.9) for quartile 2 (blood Pb 2.1-3.0µg/dL) and 2.7 (95% CI 1.2-6.2) for quartile 3 (blood Pb 3.1-4.6µg/dL) compared to quartile 1 (blood Pb 0.5-2.0µg/dL) (Nash *et al.* 2003). The odds ratios for systolic hypertension in premenopausal women were around 1.5 and not statistically significant. Other small studies found no association between blood Pb and BP or hypertension in postmenopausal women (Al-Saleh *et al.* 2005; Pizent *et al.* 2001).

Children are at greater risk of Pb exposure due to early hand-to-mouth behaviors (see further discussion in the Exposure Chapter). Few studies evaluating the effects of Pb on BP have been conducted in children (see **Table 6.5**). Young adults with childhood Pb exposure had higher bone Pb and 3-4 mmHg higher SBP and DBP (>10µg/d vs. <1 µg/g, $p<0.05$) (Gerr *et al.* 2002). In the Oswego Children's Study, cord blood Pb levels were associated with BP at age 9.5 years,

Table 6.4: Studies of Pb and blood pressure and hypertension during pregnancy used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
Supporting	705 pregnant women, USA	Prospective	Change in blood Pb concentration during pregnancy (mean 1.2µg/dL) was associated with hypertension in pregnancy, including the serious complications of preeclampsia and toxemia.	Sowers (2002)
Supporting	3,851 women at birth of child, USA	Cross-sectional	There was an increase in women's BP during labor with increased umbilical cord blood Pb, as well as an increased risk of pregnancy hypertension from low Pb levels (6.3µg/dL), but not preeclampsia.	Rabinowitz (1987)
Pregnant women, Los Angeles, USA				
Supporting	1,627 pregnant women	Cross-sectional	In the third trimester of pregnancy, increased blood Pb was associated with increased BP only in immigrant women, primarily Latina (mean blood Pb of 2.3µg/dL).	Rothenberg (1999)
	667 third trimester or postpartum women	Cross-sectional	Postpartum calcaneus bone Pb was associated with increased BP and hypertension during the third trimester (mean blood Pb 2.3µg/dL postpartum).	Rothenberg (2002)
Supporting	971 pregnant women, EDEN Study, France	Cross-sectional	Blood Pb levels in the second trimester (mean 1.9µg/dL) were correlated with BP before and after 36 weeks gestation and increased the risk of pregnancy induced hypertension, particularly in parous women.	Yazbeck (2009)
Supporting	285 pregnant women, Baltimore THREE Study, USA	Cross-sectional	Umbilical cord blood Pb of the child was associated with increased BP in the mother during labor and delivery (Q4 ≥ 0.96µg/dL vs. Q1 ≤ 0.46µg/dL), but not with other BP related pregnancy conditions.	Wells (2011)
Supporting	143 third trimester primigravid women, Malta	Cross-sectional	Blood Pb during the third trimester was higher in pregnant women with gestational hypertension (mean 9.6 vs. 5.8µg/dL) and correlated with SBP and DBP in all pregnant women.	Magri (2003)
Supporting	110 pregnant women half with gestational hypertension, Iran	Case-control	Blood Pb at delivery was higher in women with gestational hypertension (5.7 vs. 4.8µg/dL), but Pb levels did not correlate with BP in the hypertensive women.	Vigeh (2004)

Epidemiological studies of Pb exposure and blood pressure and hypertension during pregnancy listed by study type and decreasing size, grouped together for overlapping or shared study population.

BP, SBP, DBP: Blood Pressure, Systolic Blood Pressure, Diastolic Blood Pressure

Table 6.5: Studies of childhood Pb exposure and BP				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
Oswego Children's Study, USA				
Supporting	122 children	Prospective	Cord blood Pb levels (mean 3µg/dL) were associated with increased SBP at age 9.5, while childhood Pb levels (mean 4.6µg/dL at age 2.6) were associated with an increased DBP, decreased stroke volume and increased total peripheral resistance response to acute stress.	Gump (2005)
	122 children	Prospective	Family socioeconomic status interacted with blood Pb to increase BP and may interact with blood Pb to increase total peripheral resistance response to acute stress tasks in these children with low blood Pb (mean 4.6µg/dL at age 2.6).	Gump (2007)
	140 children, age 9-11	Cross-sectional	Concurrent blood Pb (median 0.94µg/dL) was not associated with BP or BP responses to acute stress in these children, but blood Pb was associated with measures of impaired cardiac function in response to acute stress tasks.	Gump (2011)
Not Supporting	780 children with moderately high Pb, half treated with succimer, USA	Cross-sectional	In these children with Pb exposure (20-44µg/dL) there was no association between blood Pb and BP, including after 5 years of follow up when mean blood levels were 8µg/dL. Chelation of Pb with succimer had no effect on BP.	Chen (2006)
Supporting	508 young adults, half lived near Pb as children, USA	Cross-sectional	In young adults, some of whom had childhood Pb exposure, SBP and DBP was significantly increased in those with the highest bone Pb levels (>10µg/g, blood Pb = 3.15µg/dL).	Gerr (2002)
Not Supporting	144 children in the town unexposed to Pb; Pristina, Kosovo	Cross-sectional	For the children residing in the town without Pb exposure, the small positive correlation of blood Pb with BP was not statistically significant.	Factor-Litvak (1996)

Epidemiological studies of childhood Pb exposure and blood pressure and hypertension listed by study type and decreasing size, grouped together for overlapping or shared study population.
BP, SBP, DBP: Blood Pressure, Systolic Blood Pressure, Diastolic Blood Pressure

and early childhood blood Pb (mean age 2.6 years) was associated with increased BP in response to acute stress tasks at age 9.5 years – particularly in children with low socioeconomic status (Gump *et al.* 2007; Gump *et al.* 2005). Other studies of blood Pb in children did not find an effect on BP (Chen *et al.* 2006; Factor-Litvak *et al.* 1996; Factor-Litvak *et al.* 1999). The adult origin of disease from childhood Pb exposure has not been sufficiently studied to inform a conclusion on cardiovascular risks from early or chronic Pb exposure.

While most of the literature supports a role for Pb in risk of hypertension the definition of hypertension is not consistent across studies. The current standard is ≥140 mmHg SBP and/or ≥90 mmHg DBP, but several studies used a higher (Apostoli *et al.* 1990; Bakhtiarian *et al.* 2006; Elmarsafawy *et al.* 2006; Grandjean *et al.* 1989), lower (Al-Saleh *et al.* 2005), or "borderline" (140-159 mmHg SBP and/or 91-94 mmHg DBP) (Staessen *et al.* 1996) definition of hypertension. Studies also differed by how subjects taking anti-hypertensive medication were included, with one study excluding these subjects from analyses of BP (Scinicariello *et al.* 2010) and several considering subjects hypertensive based only on medication use (Al-Saleh *et al.* 2005; Hu *et al.* 1996; Martin *et al.* 2006; Muntner *et al.* 2005; Nash *et al.* 2003; Scinicariello *et al.* 2010; Staessen *et al.* 1996).

Summary of Support for Conclusions on BP and Hypertension

Animal studies provide strong evidence for low level Pb elevating BP in humans and contributing to the onset of hypertension, even after the exposure to Pb has stopped (ATSDR 2007 pg 28; U.S. EPA 2006 pg 5-103). In rats, blood Pb levels as low as 2.15µg/dL showed significant increases in both SBP and DBP compared to unexposed rats, while exposures resulting in blood Pb levels over 29µg/dL did not show further increases in BP (Tsao *et al.* 2000). Many in vivo and in vitro studies support oxidative stress as the mechanism by which Pb contributes to the pathogenesis of hypertension (see U.S. EPA 2006 for further review of animal and mechanistic studies). Experimental animals do not show consistent dose-dependent increases in risk of hypertension with low, but not high, levels causing hypertension in some models (U.S. EPA 2006 pg 5-124). This lack of a monotonic relationship could partially explain inconsistency observed in human studies where studies with mean blood Pb levels below 5µg/dL were generally more supportive of a relationship with BP and hypertension (see Blood Pressure (BP) and Hypertension Section of the Cardiovascular Appendix).

The conclusion of *sufficient* evidence for a Pb-related increase in BP and risk of hypertension is based on a large body of literature in humans that is more consistently found with bone Pb as a measure of exposure than with blood Pb. There is a small but *sufficient* literature supporting Pb-related increases in hypertension during pregnancy, while there is *inadequate* evidence for Pb effects on BP or hypertension in children. The NTP's conclusions for *sufficient* evidence for blood pressure and hypertension at blood Pb levels <10µg/dL, expands the conclusions of EPA's 2006 AQCD for Pb (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007).

6.3.2 Heart Rate Variability

There is *inadequate* evidence to evaluate a potential association between Pb exposure and heart rate variability (HRV). HRV reflects sympathetic (low frequency only) and parasympathetic (high and low frequency) autonomic nervous system function, with decreases in variability indicating abnormal autonomic function (Park *et al.* 2006). The literature contained only four publications of Pb and HRV with mean blood Pb levels below 10µg/dL, and the results were not consistent (Gump *et al.* 2011; Jhun *et al.* 2005; Park *et al.* 2008; Park *et al.* 2006). In the Oswego Children's Study, concurrent blood Pb in children age 9-11 years (median blood Pb 0.94µg/dL) was significantly associated with impaired autonomic response to acute stress tasks as evaluated by HRV (Gump *et al.* 2011). In adults there was an indication that Pb may modify the effect of other metals (Jhun *et al.* 2005) or air pollution (Park *et al.* 2008) on HRV. The EPA's 2006 AQCD for Lead (U.S. EPA 2006) mentions HRV as a possible intermediary between Pb exposure and cardiovascular mortality and it is not considered in the ATSDR's Toxicological Profile for Lead (ATSDR 2007).

6.3.3 Electrocardiogram Abnormalities

There is *limited* evidence for Pb effects on electrocardiogram (ECG) abnormalities. The Normative Aging Study supports a role for bone Pb and ECG abnormalities at the time of Pb measurement and eight years later for QT and JT/ST prolongation and intraventricular or atrioventricular conduction defects (Cheng *et al.* 1998; Eum *et al.* 2011). Polymorphisms in iron metabolism genes (*HFE*, *TFC2*, *HMEX-1*) may modify these relationships (Park *et al.* 2009a). The Oswego Children's Study found that early childhood blood Pb and concurrent blood Pb were associated with decreased stroke volume and increased total peripheral resistance in response to acute stress tasks at age 9.5 years, especially in subjects with lower socioeconomic status who had higher blood Pb (overall mean 4.6µg/dL at age 2.6) (Gump *et al.* 2011; Gump *et al.* 2007; Gump *et al.* 2005). The studies of ECG abnormalities by Cheng et al. (1998) and Gump et al. (2005) were included in the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and the ATSDR's Toxicological Profile for Lead (ATSDR 2007), but no conclusions were reached.

6.3.4 Clinical Cardiovascular Disease

There is *limited* evidence that blood Pb levels <5 µg/dL are associated with clinical cardiovascular disease (see **Table 6.6** and Clinical Cardiovascular Disease Section of the Cardiovascular Appendix). A positive association has been reported between blood Pb level and several related cardiovascular diseases including peripheral arterial disease, coronary artery disease, as well as blood flow measures indicative of atherosclerotic vascular resistance.

For cardiovascular disease in general, particularly conditions exacerbated by increases in BP, there was an increased risk from Pb exposure. In large studies that found a relationship between Pb and BP, Pb was also associated with an increased incidence of coronary artery disease (NAS: (Jain *et al.* 2007)) and prevalence of peripheral artery disease (NHANES: (Guallar *et al.* 2006; Muntner *et al.* 2005; Navas-Acien *et al.* 2004)), while in the Glostrup Population Study there was no association between Pb and BP or cardiovascular disease (Moller and Kristensen 1992).

Table 6.6: Studies of Pb and clinical cardiovascular disease used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
Not Supporting	1,052 adults age 40+ Glostrup Population Study, Denmark	Prospective	After 14 years of follow-up blood Pb levels dropped, but there was no association with **coronary heart disease** or **cardiovascular disease** (fatal and non-fatal cases) in adults with 11.5µg/dL mean blood Pb at baseline aged 40 years in 1976.	Møller (1992)
Supporting	837 men, NAS, USA	Prospective	Blood and patella bone Pb were associated with an increased risk of **coronary heart disease** later in life in these older men with low blood Pb levels (mean 6.3µg/dL).	Jain (2007)
Not Supporting	13,043 Pb workers, Special Health Surv-eillance Program, South Korea	Cross-sectional	Pb workers showed no increased risk of **coronary heart disease** or **cerebral vascular disease** at low levels of exposure (5-10µg/dL vs. <5µg/dL).	Kim (2008)
NHANES (≥ 1999)				
Supporting	9,961 adults, 1999-2002	Cross-sectional	**Peripheral artery disease** prevalence and risk increased with increasing quartiles of blood Pb (highest ≥2.47µg/dL) in these general population adults.	Muntner (2005)
	4,447 adults over 40, 1999-2002	Cross-sectional	Blood Pb was associated with **peripheral artery disease** in these older adults with low levels (mean 1.95µg/dL) after adjustment including renal function.	Guallar (2006)
	2,125 adults over 40, 1999-2000	Cross-sectional	These older subjects with **peripheral artery disease** had higher blood Pb, the risk of **peripheral artery disease** increased with increasing Pb (highest quartile: >2.9µg/dL).	Navas-Acien (2004)
Supporting	420 male bus drivers, Bangkok, Thailand	Cross-sectional	**Aging index of second derivative finger photoplethysmogram waveform** (SDPTG-AI), an assessment of arterial properties, is correlated with blood Pb in male bus drivers (mean 6.3µg/dL) and may be an independent cardiovascular risk factor.	Kaewboonchoo (2010)
Supporting	197 women, Athero-sclerosis Risk Factors in Young Females Study, Austria	Cross-sectional	**Intima-media thickness of the common and carotid arteries** was increased at very low levels of Pb (highest tertile = >0.82µg/dL).	Zeller (2010)
Supporting	128 ceramic painters, Japan	Cross-sectional	In these ceramic painters increases in blood Pb were associated with decreases in postural changes in **finger blood flow volume** consistent with an atherosclerotic effect, although the majority of the subjects had Pb levels above 10µg/dL and other cardiac function tests were not associated.	Ishida (1996)
Supporting	130 MI patients, 61 controls, Pakistan	Case-control	Patients admitted for **myocardial infarction** (MI) had higher hair Pb levels, increasing for 2nd and 3rd MI, and survival of 3rd MI decreased with higher hair Pb.	Afridi (2010)

Epidemiological studies of Pb exposure and cardiovascular morbidities listed by study type and decreasing size, grouped together for overlapping or shared study populations

NHANES: National Health and Nutrition Examination Survey

NAS: Normative Aging Study in the Boston area, began in 1963 by the Veterans Administration

In prospective analyses, the NAS reported an increased risk of coronary heart disease (MI or angina) with blood Pb (Log blood Pb adjusted HR=1.45, 95% CI 1.01-2.06, p=0.05) and bone Pb (Log patella Pb adjustedHR=2.64, 95% CI 1.09-6.37, p=0.05) (Jain *et al.* 2007). The prospective Glostrup Population Study in Denmark with a mean blood Pb at baseline of 11.6µg/dL failed to find an increased risk of coronary heart disease or cardiovascular disease (Moller and Kristensen 1992).

A large cross-sectional study in Korea with low blood Pb (geometric mean 6µg/dL) found no increased risk for coronary heart disease or cerebral vascular disease (Kim *et al.* 2008). Two cross-sectional studies with mean blood Pb levels >10µg/dL do support a relationship between blood Pb and coronary heart disease and stroke (Pocock *et al.* 1988; Schwartz 1991). In cross-sectional studies of peripheral artery disease, risk increased with blood Pb in NHANES 1999-2002 studies with very low Pb levels (mean 1.6-2.1µg/dL) (Guallar *et al.* 2006; Muntner *et al.* 2005; Navas-Acien *et al.* 2004), independent of an unadjusted association with homocysteine level and accounting for renal function (Guallar *et al.* 2006).

Several studies used vascular measures as indicators of arterial function in the absence of physician diagnosed disease, but there was inadequate information to form a conclusion as each was only studied for an association with Pb exposure in one population. In a study of Japanese ceramics workers, blood Pb (mean 13µg/dL) was associated with decreased finger blood flow in response to a postural change, consistent with an atherosclerotic effect; but four other measures of cardiac function were not associated (Ishida *et al.* 1996). In a study of bus drivers with low blood Pb levels (mean 6µg/dL), mean aging index of second derivative finger photoplethysmogram waveform (SDPTG-AI) was increased with blood Pb, indicating lower central and peripheral arterial function (Kaewboonchoo *et al.* 2010). In otherwise healthy young women with very low blood levels (mean not reported, but almost all subjects <1µg/dL), intima-media thickness of the common and carotid arteries was increased with blood Pb, while there was no association with increases in eight other metals tested (Zeller *et al.* 2010). The associations of blood Pb with these tests of cardiovascular functions have not been replicated, but they suggest a role for Pb in early hallmarks of impaired cardiovascular function without a diagnosis of clinical cardiovascular disease.

Summary of Support for Conclusions on Clinical Cardiovascular Disease

The EPA's 2006 AQCD presents a small animal literature supporting an atherogenic effect of chronic Pb exposure, as well as impacts on vascular tissue and smooth muscle cells (see U.S. EPA 2006 for further review of these studies). They reported effects of Pb as conducive to thrombosis, hyperlipidemia, arteriosclerosis, and vascular remodeling. The human data are consistent with these findings, but diverse in scope - hampering the ability to form conclusions on specific cardiovascular disease endpoints.

TheNTP's conclusion of *limited* evidence for clinical cardiovascular disease is based on a heterogeneous group of related cardiovascular outcomes in studies that mostly found significant effects associated with blood Pb <5µg/dL.

6.3.5 Cardiovascular Mortality

There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with increased mortality from cardiovascular causes (see **Table 6.7** and Cardiovascular Mortality Section of the Cardiovascular Appendix). A positive association between cardiovascular mortality and blood lead was supported by three prospective studies, but not supported by two prospective studies, one of which reported a significant association with bone Pb. One of the supportive studies had low mean blood Pb levels (2.58µg/dL) (Menke *et al.* 2006), but further conclusions on risk from levels below 10µg/dL could not be drawn.

Large studies that found associations between Pb and BP also indicated an increased risk of mortality from cardiovascular causes. In NHANES III, after 12 years of follow-up using the National Death Index, there was increased mortality associated with baseline blood Pb levels (Menke *et al.* 2006; Schober *et al.* 2006). In the Normative Aging Study, bone Pb, but not blood Pb, was associated with BP and cardiovascular mortality (Weisskopf *et al.* 2009). In the Glostrup Population Study, there was no association between blood Pb and BP, cardiovascular disease, or total mortality after 14 years of follow-up (Moller and Kristensen 1992). While Pb levels were not associated with incidence of myocardial infarction (MI), subjects who died of a third MI had significantly higher hair Pb levels (Afridi *et al.* 2010).

Summary of Support for Conclusions on Cardiovascular Mortality

Mortality from cardiovascular causes is not addressed in the EPA's 2006 AQCD summary of the animal data. The conclusion of *sufficient* evidence for Pb in cardiovascular mortality is based on the three studies with a significant effect of blood Pb and one of bone Pb. The NTP's conclusions for *sufficient* evidence for cardiovascular mortality at blood Pb levels <10µg/dL expands upon the conclusion from ATSDR's Toxicological Profile which included discussion of the more general cerebrovascular mortality.

Table 6.7: Studies of Pb and cardiovascular mortality used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Cardiovascular Findings**	**Reference**
NHANES III				
Supporting	13,946 adults	Prospective	Mortality was increased with higher Pb levels for death from all causes, cardiovascular disease, myocardial infarction, and stroke – but not cancer – for these general population adults with low blood Pb levels (geom. mean=2.58µg/dL) and up to 12 years of follow-up.	Menke (2006)
	9,757 adults over 40	Prospective	In this cohort of older NHANES III participants followed for up to 12 years, blood Pb was associated with higher mortality from all causes, cardiovascular disease, and cancer, even at low levels (5-10µg/dL).	Schober (2006)
Not Supporting	1,052 adults, 14 year follow-up, Glostrup Population Study, Denmark	Prospective	In adults with 11.5µg/dL mean blood Pb at age 40, there was no association with all-cause mortality after 14 years of follow-up while there were reductions in blood Pb levels over this time.	Møller (1992)
Supporting	927 dialysis patients, Taiwan, 18 months of follow up	Prospective	In these hemodialysis patients, baseline blood Pb over 12.64µg/dL (median 16.4µg/dL) was associated with higher all-cause, cardiovascular cause, and infection cause mortality over 18 months of follow up.	Lin (2011)
Supporting	860 men in NAS with bone Pb and 1,235 with blood Pb (1994) and follow-up (2007)	Prospective	Bone Pb >35ug/g increased the risk of mortality from all causes and from and cardiovascular causes, but not cancer mortality. Blood Pb was not associated with mortality (highest >6µg/dL).	Weisskopf (2009)

Epidemiological studies of Pb exposure and cardiovascular mortality listed by decreasing size, grouped together for overlapping or shared study populations
NHANES: National Health and Nutrition Examination Survey
NAS: Normative Aging Study in the Boston area, began in 1963 by the Veterans Administration

6.4 Susceptible Populations and Modifiers of Pb Exposure

Segments of the population that are more susceptible to health effects of Pb are discussed more extensively in the Exposure chapter. It is unknown whether chronic exposure and other cardiovascular risk factors can modify the relationship between Pb and blood pressure, putting some portions of the population at greater risk of cardiovascular disease. These other factors may also impair the ability to detect an association of Pb with BP in some general population studies, even those with higher Pb levels. This concept was proposed by Orssaud *et al.* (1985) "the increase in blood lead concentration parallels the increase in blood pressure until some limit value, so that such a trend is apparent only when other factors (such as age) do not competitively increase blood pressure by greater amounts."

Susceptible Populations: Pb exposure may disproportionally impact populations with preexisting conditions exacerbated by a small increase in BP (see **Section 7.0 Renal Effects**). A prospective study of dialysis patients found significantly increased cardiovascular mortality over 18 months of follow-up for the middle Pb tertile (8.5-12.6µg/dL: HR=3.7, 95% CI 2.1-6.5) and high Pb tertile (>12.6µg/dL: HR=9.7, 95% CI 2.1-23.3) compared to the low Pb tertile (<8.5µg/dL) (Lin *et al.* 2011).

As discussed in **Section 6.3.1 Blood Pressure (BP) and Hypertension**, pregnant women, menopausal women, and children may be at greater risk of Pb related increases in BP (see further discussion in **Section 3.4 Modifiers of Pb Exposure**). The evidence for an effect of Pb on BP in children is limited, but blood Pb at age 2 was also associated with ECG abnormalities at age 9.5 years, particularly in subjects with lower socioeconomic status (Gump *et al.* 2007; Gump *et al.* 2005).

Modifiers: Age is associated with higher BP and increased risk of cardiovascular disease, and Pb levels are also strongly correlated with age (Den Hond *et al.* 2002). It is unclear if this is a cohort effect of higher Pb exposures in earlier eras or if the elderly are a population more susceptible to cardiovascular effects of Pb (see further discussion in Section 1.3 Modifiers of Pb Exposure in the Exposure Chapter). Many studies adjusted for age in their analyses, while others performed stratified analyses within age subgroups. One study of subjects over age 75 found no association between blood Pb and BP (Nordberg *et al.* 2000).

Gender and ethnicity are also correlated with Pb exposure and cardiovascular risk and frequently adjusted for in analyses. Many general population studies found higher blood Pb, higher BP, and more hypertension in non-Caucasian populations (Den Hond *et al.* 2002; Rothenberg *et al.* 1999; Scinicariello *et al.* 2010). Men generally have higher blood Pb and BP than women (Chu *et al.* 1999; Den Hond *et al.* 2002; Hense *et al.* 1993, 1994; Staessen *et al.* 1990). Many occupational studies only included male subjects even in those with low occupational Pb exposure (mean Pb levels <10µg/dL) (Kaewboonchoo *et al.* 2007; Orssaud *et al.* 1985; Schuhmacher *et al.* 1994; Sharp *et al.* 1990; Sirivarasai *et al.* 2004; Wolf *et al.* 1995), while only the Nurses' Health study focused on female workers (Korrick *et al.* 1999). Numerous other studies included gender in their adjustment factors.

Genetic variation is an important biological parameter that can characterize a susceptible population and modify the relationship between an exposure and disease via altered absorption or metabolism. Unlike other relevant cofactors, genotype is almost always blinded from the subject and investigator and is less likely to bias exposure or ascertainment. In the three studies that included genetic variants in evaluation of Pb and cardiovascular outcomes, the genotyped polymorphisms were not independent risk factors for increased pulse pressure, hypertension, or QT prolongation; but carriers of genetic variants had stronger Pb-outcome associations (Park *et al.* 2009a; Scinicariello *et al.* 2010; Zhang *et al.* 2010). These genetic modifications of the effect of Pb on cardiovascular outcomes have not been sufficiently studied to judge their reproducibility. However, if genetic variants can modify the impact of Pb exposure, it supports a biological basis for Pb-outcome associations beyond what might spuriously arise by unmeasured confounding factors.

Dietary factors may also modify the relationship between Pb and BP. People who drank alcohol had higher blood Pb and stronger associations between Pb and BP (Hense *et al.* 1994; Menditto *et al.* 1994; Pizent *et al.* 2001). Among black male bus drivers, infrequent caffeine users had a stronger positive relationship between blood Pb and BP than habitual users (Sharp *et al.* 1990). There is some indication from the literature that there is an interaction between Pb and calcium intake (Dolenc *et al.* 1993) with low calcium having higher blood Pb (Pizent *et al.* 2001) and a higher risk of hypertension (Elmarsafawy *et al.* 2006). However, a 12-week calcium supplement intervention had no effect on blood Pb, indicating any effect of calcium on BP is not via interaction with Pb (Morris *et al.* 1990).

6.5 Conclusions

The NTP concludes that there is *sufficient* evidence for a small, but detectable, increase in BP associated with Pb exposure in populations with mean blood Pb levels <10µg/dL (see **Table 6.8** for complete list of cardiovascular effects conclusions). There is *sufficient* evidence for an increase in BP and hypertension during pregnancy at levels <10µg/dl. There is also *sufficient* evidence for increased mortality from cardiovascular causes from Pb levels <10µg/dL. There is *limited* evidence of an increase in early markers of impaired cardiac function (ECG abnormalities) and cardiovascular disease in general. There is *inadequate* evidence to evaluate Pb effects at levels <10µg/dl on heart rate variability, specific cardiovascular morbidities, or any cardiovascular effects in children. There is *inadequate* evidence to evaluate Pb effects at levels <10µg/dl on hypertension or other cardiovascular disease in menopausal women.

Bone Pb reflects chronic Pb exposure and is more consistently found to be associated with increased BP, hypertension, cardiovascular disease, and mortality. Not all human studies conducted at low levels of exposure support these associations. The animal data strongly supports a causative relationship, even at low levels relevant to human exposure (e.g., BP increases in rats with blood Pb as low as 2.15µg/dL Tsao *et al.* 2000). The observed heterogeneity in the human literature may be partially explained by variation in biologically susceptible groups with other Pb-related risk factors for cardiovascular disease such as age, alcohol, caffeine, and calcium intake, or with genetic polymorphisms in metabolic genes.

Menopausal women and children may also be at increased risk of cardiovascular effects from Pb exposure, but they have been inadequately studied.

Table 6.8: Conclusions on cardiovascular effects of low level Pb				
Health Effect	**Population**	**Conclusion**	**Blood Pb Evidence**	**Bone Pb Evidence**
Blood Pressure and Hypertension	Adults	*Sufficient*	Yes, <10µg/dL	Yes
	Children	*Inadequate*	Unclear	Yes (one study)
	Pregnant Women	*Sufficient*	Yes, <10µg/dL	Not studied
	Menopausal Women	*Inadequate*	Unclear	Not studied
Heart Rate Variability	Adults	*Inadequate*	Unclear	Yes (one study)
Electrocardiogram Abnormalities	Men	*Limited*	No	Yes (one study)
	Children	*Limited*	Yes, <5µg/dL (one study)	Not studied
Clinical Cardiovascular Disease (General)	Adults	*Limited*	Yes, <5µg/dL	Yes (one study)
Clinical Cardiovascular Disease (Specific)	Adults	*Inadequate*	Unclear	Yes (one study)
Cardiovascular Mortality	Adults	*Sufficient*	Yes, <10µg/dL	Yes (one study)

7.0 RENAL EFFECTS

7.1 Conclusions

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5µg/dL in adults are associated with adverse effects on kidney function adults. With few exceptions epidemiological studies of the general population reported positive associations between blood Pb levels ≤10µg/dL and increased risk of chronic kidney disease and negative associations with estimated glomerular filtration rate (eGFR) and creatinine clearance. The associations are typically stronger in certain sub-populations, namely hypertensives and diabetics (Muntner *et al.* 2003; Tsaih *et al.* 2004). The NTP recognizes that an individual with blood levels <10µg/dL during adulthood may have had higher blood Pb levels earlier in life, and the role of early-life Pb exposure cannot be discriminated from the role of concurrent blood Pb. Comparatively few studies examined markers of exposure other than blood Pb levels; therefore, it is unknown if blood or bone Pb levels would be a better indicator of kidney effects.

The data are *inadequate* to evaluate whether prenatal exposure to lead is associated with impaired kidney function later in life. No studies were identified that evaluated prenatal blood Pb and kidney function in children or adults. Relatively few studies have assessed kidney measures in children in association with low-level Pb exposure. The findings from these studies are less consistent compared to studies in adults. Most of these studies also utilize kidney biomarkers whose prognostic value for renal function is less clear compared to GFR measures and biomarkers, such as microalbuminuria, commonly measured in the adult studies. Thus, there is currently *inadequate* evidence to conclude that blood Pb <10µg/dL is associated with impaired kidney function in children below 12 years of age. In contrast, there is *limited* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in children 12 and over. A recent study of children and young adults aged 12 to 20 in NHANES 1988-1994 with mean blood Pb of 1.5µg/dL reported a reduction in eGFR rate per doubling of blood Pb (Fadrowski *et al.* 2010). The reduction in GFR demonstrated in Fadrowski *et al.* (2010) is consistent with results from adults within NHANES and supports adverse effects on kidney function in children age 12 and over at blood Pb <5µg/dL.

7.2 How conclusions were reached

Conclusions in the NTP evaluation of Pb-related kidney effects in humans associated with low-level Pb are derived from epidemiological studies with a focus on blood Pb levels <10µg/dL. For this evaluation we did not consider studies with mean blood Pb above 15µg/dL, because in those studies, the subjects with Pb ≤10µg/dL are often used as the referent group and are not appropriate for evaluating low-level Pb effects. This evaluation focuses on the human data for kidney effects of Pb because there is a relatively large database of human studies for these endpoints; therefore, the document makes limited use of the data from laboratory animals to support the human evidence. Major endpoints considered as potential indicators of kidney effects of Pb are listed and briefly described in **Section 7.2.1**. This document is not a review of kidney toxicity and the reader is directed to published reviews for additional background. Key

data and principal studies considered in developing the NTP's conclusions are discussed in detail in **Section 7.3 Evidence for Pb-related Effects on Kidney Function**. The discussion of each kidney effect begins with a statement of the NTP conclusion whether the specific effect is associated with a blood Pb level <10µg/dL or <5µg/dL and the age group in which it is identified (childhood, or adulthood) as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. Although the information necessary to support the NTP's conclusions is presented in **Section 7.3**, the complete dataset of human studies considered for evaluation of kidney effects of low-level Pb is included in the Kidney Appendix and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 7.2.2** below.

7.2.1 Principal Measures of Kidney Effects

Table 7.1 lists a number of kidney endpoints commonly evaluated in epidemiological studies. The most clinically accepted measure of kidney function is glomerular filtration rate (GFR), the flow rate of filtered fluid through the kidney. The gold standard method of determining GFR is through the use of radionuclide or radiocontrast markers, which is both costly and time consuming. GFR can be approximated by creatinine clearance which compares creatinine levels in blood and urine to calculate the volume of blood plasma cleared per ml of creatinine per unit time. Direct measurement of creatinine clearance requires 24-hour urine collection. There are also a number of equations for estimating GFR or creatinine clearance based on serum biomarkers (e.g., creatinine, cystatin C) and consideration of other variables such as age, sex, race, or weight. Historically, serum creatinine has been most used most often although serum cystatin C is increasingly being used as an alternative or complimentary approach to serum creatinine for estimating GFR.

GFR is notoriously insensitive (Levey *et al.* 1999) and "early biological effect markers" (EBEs), such as N-acetyl-β-D-glucosaminidase (NAG), are thought to be more sensitive because they are often elevated when GFR measures are not abnormal. However, the validity and reliability of EBEs for long-term prognostic value is unclear. The epidemiological studies in children more often assess EBEs for kidney rather than eGFR or creatinine clearance. It should also be noted that the list of biomarkers of EBEs presented in **Table 7.1** is not comprehensive and reflects those most commonly reported in the Pb epidemiological studies. Other biomarkers of kidney function continue to be assessed within kidney epidemiology research, especially for acute kidney injury (AKI), with recent attention focusing on urinary proteins such as neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and liver-type fatty acid binding protein (Devarajan 2010; Tesch 2010).

Table 7.1: Commonly Used Indicators of Kidney Function in the Pb literature References			
Kidney endpoint	**Measurement**	**Description**	**Indication of Impaired Kidney Function**
Clinical Indicators of impaired kidney function			
glomerular filtration rate (GFR)	serum creatinine (most common)	breakdown product of creatine phosphate in muscles; commonly used measure of GFR (considered less precise than cystatin C); can be influenced by non-kidney function variables that affect muscle mass (gender, age, race, weight, diet)	↑serum concentration
	serum cystatin C	a cysteine protease inhibitor protein used as an alternative to serum creatinine or complementary measure to estimate GFR	↑ serum concentration
	eGFR (based on equations)	Modification of Diet in Kidney Disease (MDRD) Study: Clinical standard of estimated GFR based on creatinine but underestimates at levels in normal range CKD-Epidemiology Collaboration (CKD-EPI): More recent way to estimate GFR based on creatinine, better accuracy in normal range than MDRD	↓ eGFR
	creatinine clearance (based on timed urine collections)	Cockcroft-Gault: Oldest, estimates creatinine clearance	↓ creatinine clearance
	blood urea nitrogen (BUN)	measures the amount of nitrogen in blood in the form of urea (a waste product of protein digestion)	↑serum concentration
	^{125}I-iothalamate, iohexol and other radioisotopes	radioactive markers used to measure GFR by timed sequential blood samples or imaging (invasive and time consuming)	↓ urinary clearance
Indicators of early biological effect markers (EBEs)			
function	β_2-microglobulin (only validated EBE marker)		↑ urine concentration
	total protein, albumin and low to intermediate molecular weight proteins [e.g., retinol-binding protein (RBP)], Clara cell protein, transferrin]		↑ urine concentration
biochemical or histological alteration	biochemical: urinary eicosanoids, e.g., prostaglandin E_2 (PGE2), prostaglandin $F_{2\ alpha}$, 6-keto-prostaglandin $F_{1\ alpha}$ (6-keto-PGF), and thromboxane B_2 (TXB2), fibronectin		varies/not necessarily known[1] (e.g., ↓ urine PGE2, 6-keto-PGF; ↑ TXB2)
	histological: brush border antigen, fibronectin (glomerular fibrosis)		
cytotoxicity	N-acetyl-β-D-glucosaminidase (NAG)	lysosomal enzyme involved in the breakdown of glycoproteins	↑ activity
Other			
	urate/uric acid	urate is a salt derived from uric acid; can build up in the body when uric acid is not adequately metabolized, e.g., in cases of gout	↑ urine concentration

[1](Cardenas *et al.* 1993; Rose and Post 2011)

7.2.2 Principal conclusions from the 2006 EPA and 2007 ATSDR Pb documents

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both concluded that epidemiologic studies support a relationship between Pb exposure kidney effects at lower blood Pb levels (**Table 7.2**). EPA states that the majority of studies in general adult and patient populations published between 1986 and 2006 indicate that Pb, at much lower doses than those causing Pb nephropathy, acts as a cofactor with other more established renal risks to increase the risk for renal dysfunction. Other explanations, such as residual confounding or reverse causality, are not likely to account for the observed associations between Pb dose and kidney dysfunction. It should be noted that EPA is in the process of revising the AQCD, and the conclusions of the external draft (U.S. EPA 2011) are largely in line with the 2006 AQCD plus additional review of the evidence for reduced kidney function at low blood Pb.

Table 7.2: Main conclusion for kidney effects in 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

EPA 2006 AQCD: "General population studies are the most important advance in this regard. These studies provide strong evidence that renal effects occur at much lower blood Pb levels than previously recognized. These effects are clinically relevant in U.S. subpopulations who continue to have higher Pb exposure than the general population. At levels of exposure in the general U.S. population overall, Pb combined with other risk factors, such as diabetes, hypertension, or chronic renal insufficiency from non-Pb related causes, can result in clinically relevant effects. Notably, the size of such susceptible populations is increasing in the US due to obesity….. The threshold for Pb-related nephrotoxicity cannot be determined based on current data. However, associations with clinically relevant renal outcomes have been observed in populations with mean blood Pb levels as low as 2.2µg/dL.(U.S. EPA 2006)
ATSDR 2007 Toxicological Profile: "The overall dose-effect pattern suggests an increasing severity of nephrotoxicity associated with increasing PbB, with effects on glomerular filtration evident at PbBs below 20 µg/dL, enzymuria and proteinuria becoming evident above 30 µg/dL, and severe deficits in function and pathological changes occurring in association with PbBs exceeding 50 µg/dL."(ATSDR, 2007 pg 79)

7.3 Evidence for Pb-related Effects on Kidney Function

7.3.1 Kidney Effects in Adults

There is *sufficient* evidence available for an association between current blood Pb levels <5µg/dL in adults, measured at the time of the study, and reduced kidney function in general populations (**Table 7.3,** see also Appendix A). Associations between low-level blood Pb and impaired kidney function have been reported in studies assessing participants in NHANES (Muntner *et al.* 2003; Muntner *et al.* 2005; Navas-Acien *et al.* 2009), the Normative Aging Study (Kim *et al.* 1996; Payton *et al.* 1994; Tsaih *et al.* 2004), the Swedish Women's Health in the Lund Area (WHILA) study (Akesson *et al.* 2005), and aboriginal and non-aboriginal residents in rural Taiwan (Lai *et al.* 2008). The blood lead levels associated with kidney effects in these studies were ≤10 µg/dL and less than 5µg/dL in the NHANES and WHILA studies.

Table 7.3: Studies of kidney outcomes in adults				
Relevance to conclusions	**Study Description***	**Study Design**	**Key Kidney Findings**	**Reference**
Normative Aging Study, USA				
Supporting	Men aged 43-90 (n=744)	Cross-sectional	Ln Creatinine clearance was negatively associated with Ln blood Pb [adjβ (SE) =-0.0403 (0.0198) µg/dl; p-value= 0.0426]. Average blood Pb: 8.1 µg/dL, range <5-26µg/dL	Payton (1994)
Supporting	Men aged 34-88 n=459	Prospective	Blood lead ≤10µg/dL positively associated with concurrent serum creatinine [β(SE)= 0.060 (0.019); p=0.002], but not change in serum creatinine [β(SE)= 0.039 (0.025); p=0.13],	Kim (1996)
Supporting	Men average age of 66 y followed for 6 years (n=448)	Prospective	Significant association with change in serum creatinine with baseline blood Pb in diabetics [adjβ(SE)= 0.076 (0.023); p-value<0.05] but not non-diabetics [adjβ(SE)= 0.006(0.005); p-value = NS]. Average baseline blood Pb of 6.5 µg/dL.	Tsaih (2004)
Cadmibel, Belgium				
Supporting	Adults aged 20-88 participating in the Cadmibel study (n=1,981)	Cross-sectional	10-fold increase in blood Pb associated with reduction in creatinine clearance of 10 ml/min (female) to 13 ml/min (male) ml/min; adjOR (95%CI) for 10-fold increase in blood Pb and impaired kidney function = 3.76 (1.37, 10.4). Average (range) blood Pb of 11.4 (2.3-72.5) µg/dL in male and 7.5 (1.7-60.3) µg/dL in female	Staessen (1992)
NHANES, USA				
Supporting	Adults aged >20 years included in NHANES 1988-1994 (n=15,211 total; 4,813 hypertensives)	Cross-sectional	Increased risk in hypertensives (but not normotensives) for elevated serum creatinine [Q2 (2.5-3.8 µg/dL) versus Q1 (0.7-2.4); adjOR (95%CI) =1.47 (1.03,2.10)] and chronic kidney disease [Q3 (3.9-5.9 µg/dL) versus Q1 (0.7-2.4); adjOR (95%CI) =1.85 (1.32,2.59)]	Munter (2003)
Supporting	Adults ≥20 years of age included in NHANES 1999-2006 (n=14,778)	Cross-sectional	Risk of having a reduced GFR (defined at <60 mL/minute/1.73 m^2) was higher for blood lead of >2.4µg/dL vs ≤1.1µg/dL [adjOR = 1.56 (95% CI: 1.17,2.08); P_{trend}=<0.001]; significant trend for albuminuria (P_{trend}=<0.001)	Navas-Acien (2009)
Supporting	Adults aged 18-75 from NHANES 1999-2002 (n=9,961)	Cross-sectional	Increased risk for chronic kidney disease (GFR <60 mL/min) associated with blood Pb of >1.63µg/dL [Q3 (1.63-2.47µg/dL) versus Q1 (<1.06 µg/dL); adjOR (95%CI) =1.89 (1.09,3.30)	Muntner (2005)
Patients				
Supporting	CKD Taiwanese patients aged 25-82 years followed for four years(n=121)	Prospective	1µg/dL higher blood Pb at baseline associated with a 4.0 mL/min/1.73 m^2 reduction in eGFR over 4 years. Average (range) blood Pb = 4.2µg/dL (1 – 13.4µg/dL)	Yu (2004)
Supporting	Chronic renal insufficiency patients aged 25-80 followed for 2 years (n=202)	Prospective	Baseline chelatable Pb was significantly associated with risk for achieving an increase in serum creatinine to 1.5 times baseline [HR(95%CI) = 1.00(1.00, 1.01)]. Average blood Pb at baseline = 5.3 µg/dL (0.6 – 16.1 µg/dL). 31 patients on chelation therapy during months 24 to 51 had better GFR outcomes.	Lin (2003)
Supporting	CKD patients aged 30-80	Prospective	Baseline chelatable Pb was significantly associated with risk for achieving an	Lin (2006a)

Table 7.3: Studies of kidney outcomes in adults				
Relevance to conclusions	**Study Description***	**Study Design**	**Key Kidney Findings**	**Reference**
	followed for 2 years		increase in serum creatinine to 1.5 times baseline [HR(95%CI) = 1.03(1.00, 1.07)]. Average blood Pb at baseline = 2.9 µg/dL (0.8 – 10.3 µg/dL). 16 patients on chelation therapy during months 24 to 51 had better GFR outcomes.	
Supporting	diabetes patients aged 33-79 followed for 1 years	Prospective	Baseline chelatable Pb was significantly associated with risk for achieving an increase in serum creatinine to 1.5 times baseline [HR(95%CI) = 1.01(1.01, 1.02)]. Average blood Pb at baseline = 6.5 µg/dL (1.9 – 19.1 µg/dL). 15 patients on 3-month chelation therapy during months 13-24 had better GFR outcomes.	Lin (2006b)
Other				
Supporting	Adult women from Women's Health in the Lund Area (WHILA) study in Sweden; n=820	Cross-sectional	Negative association with GFR [β (95%CI) = -0.20 mL/min (-0.32, -0.09)] and creatinine clearance [β (95%CI) = -0.18 mL/min (-0.30, -0.06)]. Mean (5-95% percentiles) blood Pb = 2.2 (1.1-4.6) µg/dL. *No association with NAG or α1-microglobulin*	Akesson (2005)
Supporting	Adult aboriginal and non-aboriginal men and women in rural Taiwan (n=2565)	Cross-sectional	Increased risk of serum creatinine >1.2 mg/dL for blood Pb >7.5 versus ≤ 7.5 µg/dL [adjOR = 1.92 (95%CI: 1.18-3.10)]	Lai (2008)
Not supporting	Adults aged 18-51 living near two smelters in France; n=300 and 300 age/gender matched referents	Cross-sectional	*No difference in any kidney parameters in adults living in reference (average blood Pb = 7.1 µg/dL male; and 4.2 µg/dL female) and polluted areas (average blood Pb = 6.8 µg/dL male; and 5.3 µg/dL female)* [serum creatinine, total protein, albumin, transferrin, β2-microblobulin, RBP, brush border antigen, and NAG].	de Burbure (2003)
Equivocal	Men aged 40-59 participating y in the Regional Heart Study in England (n=7,364)	Cross-sectional	Blood Pb associated with log transformed serum urate (β=0.06) and serum urea (β= –0.05); no association with serum creatinine. Blood lead levels ranged from <12.4 to 37.3 µg/dL;*Author's considered the magnitude of the changes to be small and unlikely to be of biological importance	Pocock (1984)
Equivocal	Adult London civil servants in England aged 37-58 y (n=531)	Cross-sectional	In male, significant correlation between serum creatinine and log blood Pb (r = 0.10, p=0.04). In female, no correlation with serum creatinine and log blood Pb (r=0.03, p= NS)	Staessen (1990)
Not supporting	Men aged 25-38 in Egypt classified by smoking status (n=35 smokers, 33 non-smokers)	Cross-sectional	No significant correlations were found between Pb and markers of kidney damage in smokers (14.4 µg/dL) or non-smokers (10.2 µg/dL)	Mortada (2004)
Epidemiological studies of blood Pb exposure and kidney function listed by study population and sorted whether the study conclusion was supportive, equivocal, or not supporting.				

There is no apparent threshold for kidney effects. Significant increases in risk of chronic kidney disease (CKD) based on an eGFR <60 mL/min/1.73 m^2 have been reported in NHANES for blood Pb levels of >1.63 µg/dL [adjustedOR =1.89 (95%CI: 1.09, 3.30) (Muntner *et al.* 2005)] and >2.4µg/dL [adjustedOR = 1.56 (95% CI: 1.17, 2.08) (Navas-Acien *et al.* 2009)]. Similarly, blood Pb levels of ≤5 µg/dL in the WHILA study (median 2.2, 5^{th} to 95^{th} percentile 1.1-4.6µg/dL) were significantly associated with reduced eGFR [adjβ = -0.2(95%CI: -0.32,-0.09) (Akesson *et al.* 2005)]. No significant associations with blood Pb and serum creatinine or creatinine clearance were observed in a study of 709 men in the Normative Aging Study (β coefficients not reported) (Wu *et al.* 2003); however, this study did report a significant negative association between patella Pb and creatinine clearance. Blood Pb levels were negatively associated with creatinine clearance in WHILA participants [adjβ = -0.18(95%CI: -0.3,-0.06)]. The EPA's 2006 AQCD for Lead (U.S. EPA 2006) considered the clinical significance of these findings. An increase in blood Pb reported in Akesson *et al.* (2005) of 3.5 µg/dL from the 5^{th} (1.1µg/dL) to the 95^{th} (4.6 µg/dL) percentile had the same impact on glomerular filtration as an increase in age of 4.7 years or 7 kg/m^2 in BMI (U.S. EPA 2006). A ten-fold increase from 1 to 10µg/dL would result in a 16.2 mL/min decrease in estimated creatinine clearance (U.S. EPA 2006). As discussed below under "susceptible subpopulations," the impacts of Pb on kidney function in diabetics, hypertensives, or people with chronic kidney disease from non-lead related causes are expected to be higher (U.S. EPA 2006).

Many of the studies that support an association between blood Pb and kidney outcomes included statistical adjustments for factors such as age and sex, with studies based on the Normative Aging Project, NHANES, WHILA, and rural Taiwanese populations including additional variables for smoking status and/or alcohol consumption. The significant associations remaining after adjustment suggest that these factors were not sufficient to account for the observed associations between blood Pb and kidney outcomes (Akesson *et al.* 2005; Kim *et al.* 1996; Lai *et al.* 2008; Muntner *et al.* 2003; Muntner *et al.* 2005; Navas-Acien *et al.* 2009; Payton *et al.* 1994; Tsaih *et al.* 2004). Most of these studies also included blood pressure or hypertension status as an adjustment factor, which is expected to underestimate the association between Pb exposure and kidney effects (i.e., bias towards the null) given the positive relationship that exists between Pb and blood pressure in the general population (U.S. EPA 2006). The studies that were considered equivocal or not supportive of an association typically assessed kidney effect by measurement of serum creatinine and did not consider the impact of potential or modifying variables at all (de Burbure *et al.* 2003) or to the same extent as the studies cited above that assessed GFR or creatinine clearance (Mortada *et al.* 2004; Pocock *et al.* 1984; Staessen *et al.* 1990). An additional limitation to interpretation of the non-supportive findings of de Burbure *et al.* (2003) is the finding that the average blood Pb levels between the "exposed" and referent groups were quite similar and actually higher in men in the referent group compared to men considered exposed based on living near a smelter for ≥ 8 years (average blood Pb in referents: male = 7.1 µg/dL; female= 4.2 µg/dL versus "exposed": male = 6.8 µg/dL; female = 5.3 µg/dL).

Most of the studies summarized in **Table 7.3,** were cited in EPA's 2006 AQCD for Lead (U.S. EPA 2006) and considered in relation to potential reverse causality, which would occur if impaired kidney excretion leads to less efficient elimination of Pb, and thus higher estimates of internal Pb exposure, resulting in a bias towards detecting associations between Pb and impaired kidney function. The EPA did not consider potential reverse causality sufficient to explain the associations between Pb and kidney outcomes (U.S. EPA 2006). The strongest data against reverse causality comes from the longitudinal study by Yu at al. (2004) of chronic kidney disease patients in Taiwan where both baseline blood and EDTA-chelatable Pb levels predicted kidney function decline over four years. Also, two publications from the Normative Aging Study report associations between blood Pb and serum creatinine across the entire range of serum creatinine (Kim *et al.* 1996; Tsaih *et al.* 2004), including at levels in the normal range where reverse causality would not be occurring. Other evidence considered in the 2006 EPA AQCD, cited as an April 12, 2006 personal communication from Agneta Åkesson to Virginia Weaver document, is that higher urine Pb was associated with lower estimated creatinine clearance in Swedish women in the WHILA study. Urinary excretion of Pb should decrease as kidney function declines.

Summary of support for conclusions

Data from animal studies provide strong support for an association of Pb-associated kidney toxicity including histopathological changes and decreased glomerular filtration rate with chronic exposure that results in high blood Pb levels (>40µg/dL in rats; seeATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). Rodent studies also support hyperfiltration, or increased glomerular filtration rate as part of an early phase of kidney injury (e.g., Khalil-Manesh *et al.* 1992). Many in vivo and in vitro studies support oxidative stress as the mechanism by which Pb contributes to the pathogenesis of kidney disease (see U.S. EPA 2006 for further review of animal and mechanistic studies). Although animal data support a role for oxidative stress and a potential role for metallothionein in kidney effects associated with Pb exposure, the animal studies are generally at higher Pb exposure levels (20-60µg/dL) than the blood Pb levels associated with decreased GFR in humans (<10µg/dL). The human data include multiple studies that reported an association between blood Pb levels <10µg/dL and increased risk of chronic kidney disease, as well as negative associations with GFR and creatinine clearance in the general population and even stronger evidence in hypertensives and diabetics. A number of studies reported effects at blood Pb levels in the 2-3 µg/dL range (e.g., Akesson *et al.* 2005; Muntner *et al.* 2005; Navas-Acien *et al.* 2009). Collectively, these data provide *sufficient* evidence that blood Pb levels <5 µg/dL are associated with adverse effects on kidney function in adults. The NTP recognizes that blood levels measured during adulthood in this range do not preclude the possibility that an individual might have had past exposure to higher blood levels. Although several studies of populations with high occupational Pb exposure have demonstrated an association between elevated bone Pb levels and serum creatinine (e.g., Weaver *et al.* 2009; Weaver *et al.* 2003; Weaver *et al.* 2005), few studies of populations with low blood Pb levels have included bone Pb or other exposure metrics Currently, the only data on bone Pb and kidney effects in the general population are from Tsaih *et al.* (Tsaih *et al.* 2004) on men followed up in the Normative Aging Study for six years. In this study, baseline tibia lead, but not patella lead, was significantly associated in diabetics with baseline serum

creatinine, follow-up serum creatinine, and changes in serum creatinine. Tibia lead was also associated with a change in serum creatinine in men with hypertension. No significant associations were reported in non-diabetics or normotensives. The associations were stronger between tibia lead compared to blood lead, either measured at baseline or follow-up.

7.3.2 Occupational Exposures

Assessment of kidney effects at higher blood Pb, such as those experienced in occupational cohorts, is beyond the scope of this evaluation, but is addressed in EPA's 2006 AQCD for Lead with an expanded discussion in EPA's current external review draft (U.S. EPA 2011) document. Historically, research in occupational settings where Pb levels are higher (≥30µg/dL) has been less consistent than findings from general population studies (U.S. EPA 2006). For example, several of these studies reported inverse associations including higher Pb dose with lower BUN, serum creatinine, and/or higher creatinine clearance. These seemingly paradoxical effects compared to findings at lower Pb exposure levels may indicate different mechanisms of Pb-mediated kidney toxicity in different subgroups, namely Pb-related hyperfiltration (U.S. EPA 2006). This is a condition where a sustained elevation in kidney filtration rate can lead to kidney damage over time. However, studies published since the EPA's 2006 AQCD Lead document more consistently report worse kidney outcomes in exposed workers (Alinovi *et al.* 2005; Garcon *et al.* 2007; Khan *et al.* 2008; Lin and Tai-Yi 2007; Patil *et al.* 2007; Sun *et al.* 2008). These more recent studies in workers at higher exposure levels also attenuate a previous concern that the kidney effects reported in the general populations are due to reverse causality, because no effects were observed in workers at higher exposure levels.

7.4 Susceptible Populations or Life stages

7.4.1 Children

There is *inadequate* evidence available to address the potential association between low-level blood Pb in children under the age of 12 and impaired kidney function, but *limited* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in children age 12 and over. Relatively few studies have addressed kidney function or serum creatinine in children compared to adults; more often EBEs are reported (**Table 7.4**, see also Appendix A). The predictiveness of the early indicator measures for impaired kidney function is not well-established, even in adults (U.S. EPA 2006). However, Fadrowski *et al.* (2010) analyzed data from 769 children and young adults 12-20 years of age in NHANES 1988-1994 and reported a reduced mean difference in cystatin C-based eGFR of -2.9 mL/min/1.73 m^2 (95% CI: –0.7, –5.0) per doubling of blood Pb in the fully adjusted model. When the data were analyzed based on categories of Pb exposure, the mean difference in eGFR was significantly reduced for the highest quartile (>2.9µg/dL) compared to the lowest quartile (<1µg/dL), with a mean difference in eGFR of -6.6 mL/min/1.73 m^2 (95% CI: –0.7, –12.6) and there was a significant trend across exposure categories, p-trend = 0.009. This cross-sectional

Table 7.4: Studies of kidney outcomes in children				
Relevance to conclusions	**Study Description***	**Study Design**	**Key Kidney Findings**	**Reference**
Supporting	769 children and young adults 12-20 years of age in NHANES 1988-1994	Cross-sectional	Reduced GFR (mL/min/1.73 m^2) associated with blood Pb of >2.9µg/dL versus <1µg/dL [β (95%CI) = -6.6 (–0.7, –12.6)]	Fadrowski (2010)
Supporting	Children aged 17 years living near chemical-industry areas in Belgium; n=100 Pb and 100 referent	Cross-sectional	↑ Levels of serum cystatin-C and β2 microglobulin in children with higher blood Pb (mean= 2.7µg/dL) compared to referents (mean= 1.5µg/dL)	Staessen (2001)
Supporting	Children aged 1-6 years of workers in Pakistani lead smelters and battery recycling plants; n=123 Pb and 123 referent	Cross-sectional	↑ levels of serum creatinine and urea in Pb-exposed children compared to controls (median Pb = 8.1 and 6.7 µg/dL; respectively; p ≤ 0.01 for both measures in unadjusted analyses)	Khan (2010)
Equivocal	Children aged 12-15 years in Czech Republic living near two smelters; n=91 area #1, 53 area #2, and 51 in referent site	Cross-sectional	↑Levels of urinary levels of β_2-microglobulin, Clara cell protein, and NAG in children living in area #1 (x̄ blood Pb: male= 10.9 µg/dL; female = 9.44 µg/dL) compared to referents (x̄ blood Pb: male= 8.7 µg/dL; female = 8.39 µg/dL), *but not area #2 where blood Pb levels were highest* (x̄ blood Pb: male= 14.9 µg/dL; female = 12.9 µg/dL); ↑ levels of retinol binding protein in children living in both smelter areas. Significant correlation between urinary excretion and blood Pb in total group (partial r^2=0.046, regression coefficient=0.302, p=0.005)	Bernard (1995)
Equivocal	Children aged 10 years in Poland living near Pb-producing factories; n=62 and 50 referent	Cross-sectional	Altered urinary biomarkers (transferrin, 6-keto-$PGF_{1\alpha}$, NAG B, β2 microglobulin, clara cell protein, EGF, PGE_2) between 62 exposed (x̄ blood Pb = 13.3 µg/dL) and 50 control (x̄ blood Pb = 3.9 µg/dL) children; *no difference in serum creatinine or serum Clara cell protein; no difference in other urine biomarkers* (HMW, fibronectin, αGST, AAP, γGT , NAG, IAP, α_1-microglobulin, RBP, LMW, CB7, CG9, HF5, total protein, albumin laminin, LTE_4)	Fels (1998)
Not supporting	Children aged 8.5-12.3 years in France living near two smelters; n=200 and 200 matched referents	Cross-sectional	*No difference in any kidney parameters in children living in reference (*mean *blood Pb: male = 3.4 µg/dL; female= 2.7 µg/dL) and polluted areas (mean blood Pb: male = 4.2 µg/dL; female = 3.7 µg/dL)* [total protein, albumin, transferrin, β2-microblobulin, RBP, brush border antigen, and NAG]	de Burbure (2003)
Not supporting because of direction of effect	Children aged 8.5-12.3 years in Europe living near two smelters; n=364 and 352 matched referents	Cross-sectional	Negative relationship (regression coefficients) with blood lead and serum creatinine (-0.026, p=0.007), serum cystatin C (-0.056, p=0.02), and β_2-microglobulin (-0.095, p=0.01) in children living near smelters (mean Pb: male=4.2; female 3.6 µg/dL) compared to controls (mean Pb: male=3.4; female=2.8 µg/dL)	de Burbure (2006)

study presents the strongest indication to date for an association between low-level Pb and impaired kidney function in children and is restricted to children age 12 and older. It is unknown from the analysis reported in Fadrowski *et al.* (2010) whether or not the effect is driven by the older children in the study (i.e., was decreased eGFR in children ≥17 years of age responsible for the significant decline in the entire group from 12-20 years of age). The conclusion of *limited* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in children age 12 and older is based mainly on the Fadrowski *et al.* (2010) study with support provided by the consistency of the data with effects observed at similar levels in adults. These findings are also consistent with reduced eGFR reported in adults (Akesson *et al.* 2005; Muntner *et al.* 2003; Muntner *et al.* 2005; Navas-Acien *et al.* 2009). This conclusion is supported by the increased serum levels of cystatin C reported in children in Belgium (Staessen *et al.* 2001), a measure that is becoming more widely accepted and used. The existing literature does not permit a determination on relative sensitivity in children and adults.

The studies of serum creatinine and other blood or urine biomarkers present less indication of an effect of Pb on kidney function in children (**Table 7.4**). de Burbure (2006) reported a negative association between blood Pb and serum creatinine (adjusted regression coefficient = -0.026, p=0.007); however, the opposite direction of effect, an increase in serum creatinine, would be considered indicative of decreased kidney function. Alternatively, the de Burbure (2006) finding may be consistent with hyperfiltration. The data in children may be inconsistent due to a lack of reliable measures in children, differential effects by age (hyperfiltration vs. decreased clearance), or other factors. An earlier study found no difference in mean serum creatinine between 62 exposed (mean blood Pb = 13.3 µg/dL) and 50 control (mean blood Pb = 3.9 µg/dL) Polish children (Fels *et al.* 1998). Khan (2010) reported a correlation of blood Pb levels with serum creatinine (rs = 0.13; p=0.05) in a study in 123 children of workers in Pakistani lead smelters and battery recycling plants and 123 control children, ages 1-6 years. Blood Pb, serum creatinine and urea were higher in the children of workers (medians = 8.1 and 6.7 µg/dL; 56 and 52 µmol/L; and 4.5 and 4.3 mmol/L, respectively ($p \leq 0.01$ for all in unadjusted analyses). Two studies assessed associations between blood Pb and serum cystatin C and reported opposite findings (de Burbure *et al.* 2006; Staessen *et al.* 2001). Staessen *et al.* (2001) measured higher levels of serum cystatin C in 17-year old Belgian children living in a chemical industrial region ($\bar{x}$ blood = Pb 2.7 µg/dL) compared to the referent population (mean blood Pb = 1.5 µg/dL). de Burbure *et al.* (2006) found a negative association with blood Pb in 300 – 600 European children aged 8.5-12.3 years (adjusted regression coefficient = -0.056, p=0.02). The same opposing direction pattern was observed for β_2-microglobulin in these two studies. Findings on urine biomarkers are also inconsistent and difficult to interpret (Bernard *et al.* 1995; de Burbure *et al.* 2003; Fels *et al.* 1998)(**Table 7.4**). For example, Bernard *et al.* (1995) found higher urinary levels of β_2-microglobulin, Clara cell protein, and N-acetyl-β-D-glucosaminidase (NAG) in children living in one "polluted" region relative to referents, but not in another "polluted" region where average blood Pb levels were the highest. The levels of blood Pb in the comparison groups are another factor to consider when interpreting the studies in children. No differences in kidney parameters were observed in de Burbure *et al.* (2003). Although the average blood Pb levels between the "exposed" and referent groups were quite

similar in this study (mean blood Pb in 200 referents: male = 3.4 μg/dL; female = 2.7 μg/dL versus 200 children considered exposed: male = 4.2 μg/dL; female = 3.7 μg/dL), no associations were observed when stepwise multiple regression analysis was conducted on the whole group of children as well.

Several studies have assessed the impact of higher Pb exposures during childhood on kidney measures, but these studies do not necessarily provide additional clarity (Coria *et al.* 2009; Inglis *et al.* 1978; Moel and Sachs 1992; Verberk *et al.* 1996). No nephrotoxicity was reported in a group of 77 individuals in rural Chile who were assessed 10 years after being exposed to Pb-contaminated flour as children in 1996 and subsequently treated with EDTA (Pb levels measured in 1996 ranged from 37 to 87μg/dL) (Coria *et al.* 2009). Similarly, Moel *et al.* (1992) did not detect any significant differences between previously Pb-poisoned children and their siblings for serum creatinine, uric acid, and β_2-microglobulin, fractional excretion of β_2-microglobulin, urinary protein:creatinine ratio, and tubular reabsorption of phosphate. The study compared 62 patients at a Chicago lead clinic who were diagnosed and chelated between 1966 and 1972 for initial blood Pb levels >100 μg/dl to 19 age-matched siblings whose initial blood Pb levels were <40 μg/dl. Kidney pathology was documented in certain adult survivors of untreated childhood Pb poisoning in Queensland prior to its removal from paint (Inglis *et al.* 1978). It is unclear whether differences in outcomes between those studies can be attributed to use of EDTA in Coria *et al.* (Coria *et al.* 2009) and Moel et al (1992); an elevated risk of low IQ was reported in that study. An impact on IQ but not on the kidney in that study may also suggest increased reserve in the kidney compared to neurological systems. Hu *et al.* (1991) reported elevated creatinine clearance rates in 21 survivors of childhood poisoning in Boston from 1930 to 1942 compared to age-, sex-, race-, and neighborhood-matched controls (1.88 versus 1.48 mL/s per 1.73m^2). There were no differences in serum creatinine levels between subjects and controls. The creatinine clearance finding is paradoxical to results reported in adults in the general population discussed above, but is consistent with the suggestion that Pb may induce kidney hyperfiltration in certain subpopulations. Verberk *et al.* (1996) looked at a number of kidney biomarkers in 151 children 3-6 years of age living near a Pb smelter in Baia Mare, Romania. The average Pb levels in the subgroups (based on proximity to the smelter) ranged from 34.2 to 43.8μg/dL (Verberk *et al.* 1996). An increase in NAG per 100μg/dl blood Pb was reported, but there were no associations with albumin, α-1-microglobulin, RBP, or alanine aminopeptidase.

7.4.2 Hypertensives, Diabetics, and Kidney Disease Patients

The impacts of Pb on kidney function in susceptible populations such as diabetics, hypertensives, or people with chronic kidney disease are expected to be higher (U.S. EPA 2006). This pattern is apparent in several studies that conducted subgroup analyses of NHANES data and the Normative Aging Study and found that associations were stronger in NHANES participants with hypertension (Muntner *et al.* 2003) or men in the Normative Aging study with diabetes (Tsaih *et al.* 2004).

Longitudinal studies in renal disease patients or type II diabetics also indicate worse disease progression (kidney function decline) in association with higher baseline blood Pb levels (Lin *et*

al. 2003; Lin *et al.* 2006a; Lin *et al.* 2006b; Yu *et al.* 2004); see also (U.S. EPA 2006, 2011; Weaver and Jaar 2010). Although most of the patient studies are relatively small in size (ranging from 87 to 202 subjects), they are longitudinal in design with follow-up ranging from 1.1 to 3.87 years. They provide additional support for kidney effects at low-levels of blood Pb and also support the conclusion that reverse causality is not likely to account for the effects of Pb on kidney function at low levels. In the study with the longest period of follow-up, 4 years, a 1µg/dL higher blood Pb at baseline in patients with chronic renal insufficiency was associated with a 4.0 mL/min/1.73 m^2 reduction in eGFR (Yu *et al.* 2004). The average blood Pb level in the sample of 121 patients was 4.2µg/dL. In order to be eligible, patients were required to have baseline EDTA-chelatable Pb below a level thought to indicate risk for Pb-related kidney toxicity. Across these studies the decline in eGFR per 1 standard deviation increase in Pb dose at baseline per year ranged from 0.16 (Lin *et al.* 2003) to 3.87 (Lin *et al.* 2006b). The magnitude of decline in eGFR in the study with the lowest baseline blood Pb (2.9µg/dL) was 1.1 per 1 standard deviation increase in Pb dose at baseline per year (Lin *et al.* 2006b). A collection of Taiwanese patient studies included intervention arms with chelation therapy (Lin *et al.* 2003; Lin *et al.* 2006a; Lin *et al.* 2006b; Lin and Tai-Yi 2007); these studies show less kidney function decline in patients undergoing chelation therapy compared to those receiving the placebo. However, these studies are complicated to interpret because of the difficulty in distinguishing outcomes that may be due to a direct beneficial effect of the chelating agent, such as via anti-oxidation, versus those outcomes due to Pb removal. In addition, they involve small number of patients and the findings should be repeated in larger populations in multiple centers.

Thus Pb-associated reduced kidney function in healthy individuals may not necessarily result in CKD although Pb may be a risk factor for CKD in susceptible patient populations (e.g., kidney patients, diabetes, obese), older populations, or when combined with exposure to other compounds known to cause kidney damage (i.e., cadmium, mercury, arsenic, zinc) (U.S. EPA 2006).

7.5 Conclusions

The NTP concludes that there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in adults (see **Table 7.5** for complete list of kidney effects conclusions). With few exceptions epidemiological studies of the general population reported positive associations between blood Pb levels ≤10µg/dL and increased risk of chronic kidney disease and negative associations with glomerular filtration rate (GFR) and creatinine clearance. The NTP recognizes that blood levels measured during adulthood in this range does not preclude the possibility that an individual might have had past exposure to higher blood levels. As with other studies of health effects of Pb in adults, prospective studies in a population for which the data demonstrate that blood Pb levels remained consistently below 10µg/dL from birth until measurement of the outcome of interest would eliminate the potential role of early-life blood Pb levels above 10µg/dL on health effects observed in adults with concurrent blood Pb levels <10µg/dL. The associations are typically stronger in certain sub-populations, namely hypertensives and diabetics (Muntner *et al.* 2003; Tsaih *et al.* 2004). The NTP also concludes that there is *limited* evidence that blood Pb levels <5µg/dL are associated

with adverse effects on kidney function in children age 12 and older based on the recent cross-sectional data from the NHANES dataset published by Fadrowski et al. (2010) and the consistency of effects with observations in adults. There is *inadequate* evidence that blood Pb levels <10µg/dL are associated with kidney function in children under the age of 12 because of inconsistent results and studies lacking clear predictive measures of kidney function in children. The lack of consistent predictive measures of kidney function in children makes studying the effects of Pb on this life stage difficult. The NTP's conclusion that there is *sufficient* evidence that blood Pb levels <5µg/dL are associated with adverse effects on kidney function in adults and *limited* evidence in children 12 and older, extends the conclusions of the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) from adults to children age 12 and older based on recent data.

Table 7.5: NTP conclusions on kidney effects of low level Pb

Health Effect	Population	NTP Conclusions	Blood Pb Evidence	Bone Pb Evidence
Increased Chronic Kidney Disease (CKD) and decreased Glomerular Filtration Rate (GFR)	Adults	*Sufficient*	Yes, <5µg/dL	Not studied
	Children age 12 or older	*Limited*	Yes, <5µg/dL	Not studied
	Children under 12	*Inadequate*	Unclear	Not studied

8.0 REPRODUCTIVE / DEVELOPMENTAL EFFECTS

8.1 Conclusions

The NTP concludes there is *sufficient* evidence that blood Pb levels <10 μg/dL are associated with adverse health effects on development in children and reproduction in adult women.

Because the database of human studies on most reproductive endpoints is limited to occupational exposure studies, many of the available studies are for blood Pb levels >10μg/dL. Given this fact and the focus of the original nomination on reproductive and developmental effects, higher blood Pb levels were included in the evaluation of these health effects unlike other sections of this document. Consideration of blood Pb levels >10μg/dL resulted in several conclusions for Pb-related reproductive effects in men, but did not affect the conclusions for women or children.

Unlike the dataset for most other health outcomes, there are a number of prospective studies of developmental effects that include prenatal exposure metrics (either maternal blood or cord blood). Maternal blood Pb <10μg/dL is associated with decreased head circumference in children through 4 years of age providing evidence that prenatal exposure is associated with reduced postnatal growth in children. Conclusions on effects of prenatal exposure for outcomes evaluated as children are complicated by the high degree of correlation in childhood blood Pb levels over time, and as described below, concurrent blood Pb levels <10μg/dL in children are also associated with reduced postnatal growth.

Children

In children, there is *sufficient* evidence that blood Pb levels <10μg/dL are associated with delayed puberty in both boys and girls. Nine studies with mean blood Pb levels <10μg/dL support the relationship between Pb and delayed puberty (see **Table 8.3**); although several studies report effects on puberty at blood Pb levels <5μg/dL, there is also evidence indicating no effect of blood Pb <5μg/dL. Therefore, there is *limited* evidence that delayed puberty is associated with blood Pb levels <5μg/dL. There is *sufficient* evidence that decreased postnatal growth is associated with blood Pb levels <10μg/dL in children. Epidemiological studies consistently report a negative relationship between blood Pb levels below 10μg/dL and postnatal growth (see **Table 8.4**). Developmental effects on neurological, immunological, renal and cardiovascular systems are not covered in this section as they are reviewed in individual chapters.

Women

In adult women, there is *sufficient* evidence that maternal blood Pb levels <10μg/dL are associated with reduced fetal growth or lower birth weight. The association between maternal Pb exposure and reduced fetal growth is supported by several prospective studies with maternal blood Pb data during pregnancy, a large retrospective cohort of over 43000 mother-infant pairs, and a number of cross-sectional studies with maternal or cord blood Pb at delivery. Although maternal or paternal bone Pb data are not available in studies of most reproductive

health outcomes, a set of studies from a single population reported that maternal bone Pb is associated with lower birth weight, birth length, and head circumference. There is *limited* evidence that maternal blood Pb levels <10µg/dL are associated with spontaneous abortion and preterm birth or reduced gestational age. Although a number of prospective studies with maternal blood Pb levels during pregnancy and cross-sectional studies with cord blood Pb levels at delivery reported an association between prenatal blood Pb levels <10µg/dL and preterm birth, the conclusion of *limited* evidence is based on the inconsistent results and because a retrospective study with a large cohort of over 43000 mother-infant pairs did not find an association between maternal blood Pb levels and preterm birth. The conclusion of *limited* evidence for an association with spontaneous abortion in women is based principally on the Borja-Aburto *et al.* (1999) study with the strength of the prospective nested case-control design with additional support provided by occupational studies that reported an association with Pb exposure but lack blood Pb measurements. There is *inadequate* evidence for other reproductive and effects of Pb associated with blood Pb levels <10µg/dL in women.

Men

In adult men, there is *inadequate* evidence that blood Pb levels <10µg/dL are associated with effects on reproduction. There is *sufficient* evidence that blood Pb levels ≥15µg/dL are associated with adverse effects on sperm or semen in men, and *inadequate* evidence for adverse effects on sperm at lower blood Pb levels. Decreased sperm count, density, and/or concentration have been reported in multiple retrospective and cross-sectional occupational studies of men with mean blood Pb levels from 15-68µg/dL (see **Table 8.5**). There is *sufficient* evidence that paternal blood Pb levels ≥20µg/dL are associated with delayed conception time and *limited* evidence that blood Pb levels ≥10µg/dL in men are associated with other measures of reduced fertility. Four studies reported increased time to pregnancy in women whose male partners had blood Pb levels of 20-40µg/dL. A single retrospective occupational study reported increased risk of infertility among men with blood Pb levels ≥10µg/dL, and the continuity of these data with effects on time to pregnancy supports a conclusion of *limited* evidence that blood Pb levels ≥10µg/dL in men are associated with other measures of reduced fertility. There is *limited* evidence that paternal blood Pb >31µg/dL is associated with spontaneous abortion. The conclusion of *limited* evidence that spontaneous abortion is associated with paternal exposure is based mainly on the retrospective nested case-control Lindbohm *et al.* (1991a) study in men with additional support provided by occupational studies that reported an association with Pb exposure but lack blood Pb measurements.

8.2 How conclusions were reached

Conclusions in the NTP's evaluation of Pb-related reproductive and developmental effects in humans associated with low-level Pb are derived by evaluating the data from epidemiological studies with a focus on blood Pb levels <10µg/dL. Because the database of human studies on most reproductive endpoints is limited to occupational exposure studies, many of the available studies are for blood Pb levels >10µg/dL. Unlike other sections of this document, reproductive

effects of these higher blood Pb levels were included in the evaluation because there is a limited dataset of human studies to inform reproductive effects associated with lower blood Pb levels. Major endpoints considered as potential indicators of effects of Pb on reproduction and development are listed and briefly described in **Section 8.2.1**. This document is not a review of the reproductive system or reproductive and developmental toxicity and the reader is directed to published reviews for additional background. Key data and principal studies considered in developing the NTP conclusions are discussed in detail in **Section 8.3 Evidence for Pb-related Effects on Reproductive and Developmental Outcomes**. The discussion of each effect begins with a statement of the NTP's conclusion that the specific effect is associated with a blood Pb level <10µg/dL or <5µg/dL and the age group in which it is identified (childhood or adulthood) as well as the timing of exposure associated with the effect (prenatal, childhood, concurrent) when available. The discussion also highlights the extent to which experimental animal data support the association between Pb exposure and reproductive effects. Although the information necessary to support the NTP conclusions is presented in **Section 8.3**, the complete dataset of human studies considered for evaluation of reproductive and developmental effects with low-level Pb is included in Appendix E: Reproductive and Developmental Effects and individual studies are abstracted for further reference. The NTP made extensive use of recent government evaluations of health effects of Pb in the current assessment and the relevant conclusions of the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) are briefly described in **Section 8.2.2** below.

8.2.1 Principal Measures of Reproductive and Developmental Effects

Table 8.1 lists a number of key reproductive and developmental endpoints commonly evaluated in epidemiological studies. The data available to evaluate each of the major effects are discussed in separate subheadings under **Section 8.3 Evidence for Pb-related Effects on Reproductive and Developmental Outcomes** below.

Table 8.1: Major reproductive/developmental effects considered

Effect	Description
Delayed puberty	Delay in measures of puberty (e.g., Tanner genital, pubic hair, and breast staging)
Postnatal growth	Slower growth (as indicated by height, head circumference, etc. for age)
Sperm parameters	Numerous sperm or semen measures (sperm count, motility, morphology)
Conception	Greater time to pregnancy or lower fecundity
Pregnancy loss	Spontaneous abortion (fetal loss<20 weeks gestation), Stillbirth (fetal loss≥20 weeks)
Gestation length	Shorter gestation length (continuous measure)/preterm birth (<37 weeks)
Fetal growth	Lower birth weight, often adj. for gestational age
Birth defects	Congenital malformations

8.2.2 Principal conclusions from 2006 EPA and 2007 ATSDR Pb documents:

The EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's Toxicological Profile for Lead (ATSDR 2007) both concluded that there is evidence for reproductive effects in males at high blood lead levels (30-40μg/dL in ATSDR, 2007 and 45μg/dL in U.S. EPA, 2006), and suggest more research is needed to determine if effects occur at lower blood Pb levels (see **Table 8.2** for principal conclusions and original documents for complete conclusions). Although the 2006 EPA AQCD for Lead cited the Borja-Aburto *et al.* study (1999) as a well conducted prospective case-control study supporting a significant relationship between maternal blood Pb level at 10 μg/dL to 12 μg/dL and spontaneous abortion, EPA concluded that collectively there is little evidence to support an association between maternal or paternal Pb exposure and incidence of spontaneous abortion. The 2006 EPA AQCD for Lead concluded that the data are inadequate to evaluate reproductive effects in females, and studies of developmental endpoints suggest at most a small association between lead exposure and preterm birth, congenital abnormalities, birth weight, or fetal growth. EPA is in the process of revising the AQCD, and the conclusions of the external draft (U.S. EPA 2011) are largely in line with the 2006 AQCD for Lead plus additional review of the evidence for delayed pubertal onset.

Table 8.2: Main conclusion for reproductive / developmental effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"The epidemiologic evidence suggests small associations between exposure to Pb and male reproductive outcomes, including perturbed semen quality and increased time to pregnancy. These associations appear at blood Pb levels >45 μg/dL, as most studies have only considered exposure in the occupational setting. There are no adequate data to evaluate associations between Pb exposure and female fertility." (EPA, 2006 pg 6-271)
"Studies of children also have shown associations between PbB and growth, delayed sexual maturation in girls, and decreased erythropoietin production. Some studies of humans occupationally or environmentally exposed to Pb have observed associations between PbB and abortion and preterm delivery in women and alterations in sperm and decreased fertility in men. On the other hand, there are several studies that found no significant association between Pb exposure and these end points. At least for the effects in males, the threshold PbB appears to be in the range of 30–40 μg/dL." (ATSDR, 2007 pg23)

The NTP considered the conclusions and data summaries from the EPA and ATSDR documents. In general, the NTP concurred with the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints in the document.

8.3 Evidence for Pb-related Effects on Reproductive and Developmental Outcomes

8.3.1 Delayed Puberty

There is *sufficient* evidence that blood Pb levels <10 μg/dL are associated with a delay in sexual maturation or puberty onset in children aged 8-17 (**Table 8.3** and Puberty Section of Appendix E: Reproductive and Developmental Effects). A negative association between blood Pb level and markers of sexual maturation has been reported in 8 cross-sectional studies and a single prospective study involving children with blood Pb levels from 10 to less than 1 μg/dL from 7

different populations in North America, Europe, and Africa. Pb-related developmental delay in a several biological markers of puberty (e.g., age at menarche and Tanner developmental staging of breasts) have been reported in cross-sectional studies of girls, although there is no single measure that is consistently associated with blood Pb levels in all analyses. For boys, a Pb-related decrease in testicular volume was observed in all four publications, suggesting that testicular volume may be a reliable indicator of the effects of Pb on puberty in boys. The reported delay in sexual maturation with increasing blood Pb was significant across multiple studies, in various endpoints, and from different populations in analyses that adjusted for factors known to effect puberty such as race, BMI, and socioeconomic status. A conclusion of *limited* evidence that delayed puberty is associated with blood Pb levels <5 µg/dL is based on the four studies (3 in girls and 1 in boys) that report delay in markers of puberty associated with blood Pb levels below 5µg/dL and the lack of association with blood Pb among girls in the Wolff *et al.* (2008) study with a median blood Pb level of 2µg/dL.

Three cross-sectional studies utilizing the NHANES III dataset reported delayed puberty onset in girls. Wu *et al.* (2003) reported delayed Tanner pubic hair stage and age at menarche in girls aged 8 to 16 with blood Pb levels ≥2µg/dL; with no effect on developmental stage of breasts. In a separate analysis divided by race and ethnicity, Selevan *et al.* (2003) reported an association between blood Pb >3µg/dL and delayed puberty onset in African American and Mexican American girls as determined by Tanner pubic hair and breast stages. Age at menarche was also delayed at blood Pb levels >3µg/dL in African American girls; however, there was no Pb-related effect on puberty in non-Hispanic whites. Gollenberg *et al.* (2010) reported that girls with blood Pb levels ≥5µg/dL were less likely to have inhibin B levels >35 pg/ml, a level that the authors suggest is associated with pubic hair and breast development. A similar delay in puberty onset indicated by age at menarche was reported in 13-year old girls in South Africa (Naicker *et al.* 2010) and 10-17-year old girls from the Mohawk Nation (Denham *et al.* 2005). Naicker *et al.* (2010) also found an association with blood Pb ≥5µg/dL and delays pubic hair and breast development stage. In contrast, Tomoum *et al.* (2010) reported decreased Tanner breast developmental stage and no effect on pubic hair in girls aged 10-13 with blood Pb>10µg/dL in Egypt. Staessen *et al.* (2001) reported significant (p=0.04) differences in breast development in 17 year-old girls among three populations, two study groups with blood Pb levels of 1.8 and 2.7µg/dL and the referent group with blood Pb of 1.5µg/dL. However a direct comparison of breast stage by blood Pb levels was not reported and the delay in breast stage was only significant when comparing the study group with mean blood Pb of 1.8µg/dL to the referent population. One study (out of 8 studies reporting data on girls) did not detect a delay in any marker of puberty onset associated with blood Pb; mean blood Pb was 2µg/dL in the 9-year old girls from New York in that study (Wolff *et al.* 2008).

Table 8.3: Studies of biomarkers of puberty associated with low level Pb exposure used to develop conclusions				
Relevance to conclusions	**Study Description***	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Chapaevsk Russia		Prospective	Delayed puberty onset in boys with blood Pb≥5μg/dL. Puberty measures differed by testicular volume and Tanner genital and pubic hair staging.	Williams (2010) *(same population as Hauser)*
Supporting	Boys 11-12; n=481			
	Boys 8-9; n=489	Cross-sectional	Delayed puberty onset in boys with blood Pb≥5μg/dL. Puberty determined by Tanner genital staging and testicular volume; effects *not significant for pubic hair staging*.	Hauser (2008)
NHANES III		Cross-sectional	Delayed puberty onset in girls with blood Pb ≥2μg/dL compared to those with blood Pb<2μg/dL. Puberty differed by Tanner pubic hair developmental stage and age at menarche; *no effect on developmental stage of breasts*.	Wu (2003) *(population overlap with Gollenberg, Selevan)*
Supporting	Girls 8-16 years of age; n=1,235			
	Girls aged 8-11; n=705	Cross-sectional	Girls with higher blood Pb (blood Pb ≥5μg/dL compared with those with blood Pb<1μg/dL) had lower likelihood of having inhibin B level>35pg/ml, a level the authors report to be associated with puberty and development of pubic hair and breasts.	Gollenberg (2010)
	Girls aged 8-16; n=600 to 805 per race/ethnicity	Cross-sectional	Delayed puberty onset in African American girls (age at menarche, Tanner breast and pubic-hair developmental stage) and Mexican American girls (breast and pubic-hair stage) at blood Pb >3μg/dL compared to blood Pb <1μg/dL; *not in non-Hispanic whites*.	Selevan (2003)
Supporting	Girls aged 13 in South Africa; n=682 to 712 varies by endpoint	Cross-sectional	Delayed puberty onset (determined by Tanner pubic hair and breast developmental stage and age at menarche) in girls with blood Pb≥5μg/dL and significant association with blood Pb by trend analysis across for stage or age at menarche.	Naicker (2010)
Supporting	Children aged 17 in Belgium; n=100 Pb and 100 referent	Cross-sectional	Testicular volume was lower in boys living in areas with higher blood Pb (1.8-2.7μg/dL) compared to referents (1.5μg/dL). *Genital and breast stage did not differ consistently and significantly between referent and exposed*; comparison by blood Pb not reported.	Staessen (2001)
Supporting	Girls aged 10-17 in Akwesasne Mohawk Nation; n=138	Cross-sectional	Delayed puberty onset (age at menarche) in girls with blood Pb above mean (≥0.49μg/dL) and 10 month predicted delay in age at menarche with blood Pb above median (1.2μg/dL).	Denham (2005)
Not Supporting	Girls aged 9 in New York; n=139	Cross-sectional	Blood Pb had *no effect on puberty onset in girls (by breast and pubic hair stage)* with median blood Pb level of 2μg/dL.	Wolff (2008)
Supporting	Children aged 10-13 in Egypt; n=41	Cross-sectional	Delayed puberty onset in boys and girls with blood Pb≥10μg/dL. Puberty measures differed for testes size, Tanner pubic hair and penile stage in boys, Tanner developmental stage of breasts in girls; *not pubic hair in girls*.	Tomoum (2010)

* Epidemiological studies of Pb exposure and puberty listed by decreasing cohort size and grouped together for overlapping or shared study populations

All four of the studies addressing boys report Pb-associated decreases in testicular volume, Tanner genital or pubic hair staging (Hauser *et al.* 2008; Staessen *et al.* 2001; Tomoum *et al.* 2010; Williams *et al.* 2010). In a paired cross-sectional and follow up prospective study, blood Pb ≥5µg/dL was associated with delayed puberty that was significant by Tanner genital staging ($p<0.05$) and reduced testicular volume ($p\leq0.05$) in Russian boys at 8-9 years of age and when they were older (11-12 years of age) (Hauser *et al.* 2008; Williams *et al.* 2010). Testicular volume was significantly lower in 17-year old boys living in areas with higher blood levels (1.8-2.7µg/dL) compared to a referent population (1.5µg/dL); although the authors do not include a direct comparison of testicular volume by blood Pb levels (Staessen *et al.* 2001). Blood Pb ≥10µg/dL was associated with decreased testes size and developmental delay in Tanner pubic hair and penile stages in 10-13 year-old boys in Egypt (Tomoum *et al.* 2010).

Summary of support for conclusions

Animal data supports a Pb-associated developmental delay in sexual maturation indicated by biomarkers of puberty such as reduced prostate weight and delay in vaginal opening at high blood Pb levels in some studies (i.e., 40 to over 300µg/dL) in Fisher and Sprague Dawley rats and at blood Pb levels similar to the human studies (i.e., 3-13µg/dL) in Swiss mice (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The mouse data from Iavicoli *et al.* (2004) are interesting because the exposure that resulted in blood Pb from 3-13µg/dL are associated with delayed puberty in female mice similar to the human data, whereas blood Pb <3µg/dL were associated with accelerated time to puberty in mice and suggest the possibility of a different mechanism of action in mice at blood Pb levels <3µg/dL. The human data supporting Pb-associated delay in puberty include the one prospective study in boys discussed above, and are otherwise restricted to cross-sectional studies. The determination of causation from cross-sectional studies has a serious limitation because cross-sectional studies rely on concurrent blood Pb measurements and provide no information on cumulative Pb or Pb exposure at earlier time points that may be critical for sexual maturation. However, the consistency of effects across studies, among multiple populations, and in multiple measures of puberty in both males and females lends weight to the evidence for developmental delay in puberty at blood Pb concentrations from 10 to less than 1 µg/dL. The conclusion of *sufficient* evidence for developmental delay in puberty in children at blood Pb levels <10µg/dL is based on the prospective study and eight cross-sectional studies that report effects above the mean blood Pb level (0.49µg/dL to 5µg/dL) of the population under study. A lower effect level and the conclusion of *limited* evidence for delayed puberty at blood Pb<5µg/dL is supported by two of the studies that utilize the NHANES III data to examine puberty onset in girls from 8-16 years of age, the Denham *et al.* (2005) study of 10-17 year old girls in the Mohawk Nation, and the Staessen *et al.* (2003) study reporting lower testicular volume in boys. The Tomoum *et al.* (2010) study provides additional support, but reported delay in measures of puberty in boys and girls at higher blood Pb levels (i.e., ≥10µg/dL). The NTP's conclusions for *sufficient* evidence for delay in sexual maturation in boys and girls at blood Pb levels <10µg/dL and *limited* evidence at blood Pb <5µg/dL, expands the conclusion from ATSDR's 2007 Toxicological Profile for Lead which were limited to girls at blood Pb levels <10µg/dL; EPA's 2006 AQCD for Lead did not present specific conclusions on sexual maturation.

8.3.2 Postnatal Growth

There is *limited* evidence that maternal Pb <10µg/dL is associated with decreased head circumference in children up to 4 years of age and *sufficient* evidence that concurrent blood Pb <10µg/dL in children is associated with decreased postnatal growth. Prospective studies in two populations (Rothenberg *et al.* 1993; Rothenberg *et al.* 1999; Schell *et al.* 2009) report a negative association between maternal blood Pb and head circumference, and one study reports the lack of an association between maternal blood Pb and height or weight through 10 years of age (Lamb *et al.* 2008). The data from prospective studies (see **Table 8.4** and Postnatal Growth section of Appendix E: Reproductive and Developmental Effects) in two populations support a negative association between blood Pb levels in children and subsequent growth (Greene and Ernhart 1991; Rothenberg *et al.* 1993; Rothenberg *et al.* 1999). Numerous cross-sectional studies report a negative association between blood Pb in children and head circumference, height, or other indicators of growth (e.g., weight, chest circumference, etc.). The clear majority of cross-sectional studies, including studies with large sample sizes such as the NHANES datasets, demonstrate a negative association between concurrent blood Pb (means from 2 to 15µg/dL) and height or other indicators of postnatal growth in children from 1 to 16 years of age. This strong evidence for an association between growth and concurrent blood Pb is based on exposure data that is outside the relevant time window. Height is a reflection of previous growth, and the relevant Pb exposure timing is during or prior to that previous growth. Therefore, the conclusion of *sufficient* evidence that blood Pb <10µg/dL in children is associated with decreased growth is based on the combination of strong support from the cross-sectional studies and the additional support from the three prospective studies that evaluate blood Pb levels in children on subsequent growth rather than current height.

Several prospective studies support a negative association between maternal Pb <10µg/dL and postnatal growth indicated by head circumference but the relationship is less clear for height. Maternal blood Pb at 36 weeks of gestation was negatively related to head circumference up to 4 years of age in children in the Mexico City study (Rothenberg *et al.* 1993; Rothenberg *et al.* 1999). Schell *et al.* (2009) also report an effect of maternal blood Pb on head circumference, but not on height or weight in 6-12 month olds in Albany, NY. Maternal blood Pb was not related to height or weight in children from 1-10 years of age in the Yugoslavia Prospective Study (Lamb *et al.* 2008). Data from the Cincinnati Pb study support a combined effect of maternal blood Pb as well as concurrent blood Pb levels in the children; height at 15 months was only decreased in children with blood Pb >3.4µg/dL that also experienced maternal blood Pb >7.7µg/dL (Shukla *et al.* 1989). A subsequent study supported the combined effect of blood Pb at 3-15 months of age and concurrent blood Pb level for height at 33 months (Shukla *et al.* 1991). In children from the Mexico City study, infant blood Pb at 1 year of age was

Table 8.4: Studies of postnatal growth associated with low level Pb exposure used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Cincinnati Pb study		Prospective	Concurrent blood Pb (>3.4µg/dL) in children was negatively associated with growth rate (length) at 15 months of age in children of mothers with Pb (>7.7µg/dL).	Shukla (1989) *(same population as Shukla, 1991)*
Supporting	Children 15 months of age in; n= 260			
	Children ≤33 months of age; n=235	Prospective	Current blood Pb was negatively associated with length at 33 months of age in children with higher blood Pb (>10.8µg/dL) from 3-15 months of age.	Shukla (1991)
Albany Pregnancy Infancy Pb Study		Prospective	Maternal blood Pb (≥3µg/dL) was negatively associated with infant head circumference at 6 and 12 months; *not length or weight.*	Schell (2009)
Supporting	Children aged 0.5-1 in Albany; n=211			
Cleveland Pb study		Prospective	Blood Pb at 6 months of age (10µg/dL) was related to subsequent head circumference (p=0.05), and marginally related to subsequent length (p=0.06), and weight (p=0.08); blood Pb at 1-4 years of age were *not related to weight, length, head circumfer. at 4*.	Greene (1991)
Supporting	Children <5 years; n= 151-185 per sample			
Mexico City		Prospective	Maternal blood Pb at 36 weeks (median <10µg/dL) was negatively associated with infant head circumference at 6 and 18 months. Infant blood Pb (1 year) was negatively associated with head circumference at 36 months.	Rothenberg (1993) *(same population as Rothenberg, 1999)*
Supporting	Children 0.5-1 years; n=50-111 per sample			
	Children 0.5-4 year; n =119-199 per sample	Prospective	Maternal (36 weeks) and infant (1 year) blood Pb (median <10µg/dL) were negatively associated with infant head circumference at later ages up to 4 years of age.	Rothenberg (1999)
Yugoslavia Prospective Study		Prospective	Maternal blood Pb was not correlated to height or weight in children from 1-10 years of age.	Lamb (2008)
Not Supporting	Children birth, 1, 4, 6, and 10 years; n=309			
Supporting	Children at 4 year of age; n=156-175	Cross-sectional	Concurrent blood Pb (<15µg/dL) was negatively associated with height in Pristina, but blood Pb (20-40µg/dL) was not related to height in Titova-Mitrovica, a Pb smelter town	Factor-Litvak (1999)
NHANES III		Cross-sectional	Concurrent blood Pb (mean 3.6µg/dL) was negatively associated with height and head circumference; *not weight*	Ballew (1999) *(same population as Selevan)*
Supporting	Children aged 1-7; n=4391			
	Girls 8-16 years of age; n=600 to 805 per race/ethnicity	Cross-sectional	Concurrent blood Pb ≥3 µg/dL was associated with decreased height compared to individuals with a blood Pb of 1 µg/dL; *not weight*	Selevan (2003)
NHANES II		Cross-sectional	Concurrent blood Pb (range 5-35µg/dL) was negatively associated with height, weight, and chest circumference	Schwartz (1986) *(same population as Frisancho)*
Supporting	Children 0.5-7 years of age; n=2695			

Table 8.4: Studies of postnatal growth associated with low level Pb exposure used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
	Mexican-Americans age 5-12; n=1454	Cross-sectional	Concurrent blood Pb (mean 10.6 boys; 9.3µg/dL girls) was negatively associated with height	Frisancho (1991)
Supporting	Children aged 7-15 in Poland; n=899	Cross-sectional	Concurrent blood Pb (mean 7.7µg/dL) was negatively associated with height, leg length, arm length in both, trunk length in boys, weight in girls; *not weight in boys, trunk in girls.*	Ignasiak (2006)
Supporting	Children aged 2-12 in Dallas; n=764 (1980s-n=404; 2002-n=390)	Cross-sectional	Concurrent blood Pb was negatively associated with height, weight, and head circumference. The height-Pb relationship was not statistically different between children in 1980s (mean=24.8µg/dL) or 2002 (mean= 1.8µg/dL).	Little (2009)
Supporting	Children aged 7; n=602 in Mexico	Cross-sectional	Concurrent blood Pb (11.5 µg/dL) was negatively associated with height and *positively associated with head circumference*	Kordas (2004)
Supporting	Children aged 6-9 in Greece; n=522	Cross-sectional	Concurrent blood Pb (mean 12.3µg/dL) was negatively associated with height, head circumference, and chest circumference	Kafourou (1997)
Supporting	Boys 8-9 in Russia; n=489	Cross-sectional	Concurrent blood Pb (median 3µg/dL) was negatively related to height; *not weight or BMI.*	Hauser (2008)
Supporting	Children aged 11-13 in Italy; n=418	Cross-sectional	Concurrent blood Pb was negatively associated with height and weight in 13 year old boys (mean 8.5µg/dL) and height in 12 year old girls (mean 7µg/dL), not children of other ages	Vivoli (1993)
Supporting	12 month old infants in Mexico City; n=329	Prospective & Cross-sectional	Infant blood Pb (6.8µg/dL) at one month and maternal bone Pb (tibia 10.1µg/g) were negatively related to infant weight and/or weight gain through 12 months of age	Sanin (2001)
Not supporting	Children aged 6-9 in Malaysia; n=268	Cross-sectional	Concurrent blood Pb (mean 3.75µg/dL) was not correlated to height, weight, or arm circumference for age.	Zailina (2008)
Supporting	Children aged 7 and 20 in USA; n=236	Prospective & Cross-sectional	Dentin Pb level of teeth lost before age 7 was negatively associated with BMI at age 7 and BMI at age 20, *not weight or height.* no association between growth & bone Pb at age 20	Kim (1995)
Supporting	Children aged 1-10 in Dallas; n=139	Cross-sectional	Concurrent blood Pb was negatively associated with height, weight, and head circumference.	Little (Little *et al.* 1990)
Supporting	Children age 5-13 in Korea; n=108	Cross-sectional	Concurrent blood Pb (mean 2.4µg/dL) was negatively associated with height and arm length; *not weight or BMI*	Min (2008)
Not supporting	Children aged 10-13 in Egypt; n=41	Cross-sectional	Mean height and weight did not differ as a percentage of median for age and sex for individual above and below blood Pb of 10µg/dL in children with mean Pb of 9.46µg/dL.	Tomoum (2010)
Supporting	Children age 18-36 mo. in Omaha; n=21	Cross-sectional	Concurrent blood Pb (mean 6.4µg/dL) was negatively associated with head circumference	Stanek (1998)

* Epidemiological studies of Pb exposure and growth listed by decreasing cohort size and grouped together for overlapping or shared study populations

negatively related to head circumference up to 4 years of age (Rothenberg *et al.* 1993; Rothenberg *et al.* 1999). Greene *et al.* (1991) reported that blood Pb in children at 6 months of age was related to subsequent growth at borderline statistical significance for head circumference (p=0.05), length (p=0.06) and weight (p=0.08) in children in the Cleveland Pb study (n=151-185 per sample).

The clear majority of cross-sectional studies support a negative association between concurrent blood Pb (with mean levels of 2 to 15µg/dL and higher) and height. Additional measures of growth such as head circumference, arm or leg length, and weight were also related to blood Pb in some studies but these endpoints were less widely reported, and weight is less consistently related to blood Pb. Data from NHANES II support a negative association between concurrent blood Pb from 5-35 µg/dL and height, weight and chest circumference (Frisancho and Ryan 1991; Schwartz *et al.* 1986). The negative relationship between height (but not weight) and blood Pb was further supported by studies using data from NHANES III on children aged 1-7 with mean blood Pb of 4µg/dL (Ballew *et al.* 1999) and in girls aged 8-16 with blood Pb ≥3µg/dL (Selevan *et al.* 2003) compared to girls with blood Pb of 1µg/dL. Other large cross-sectional studies have reported a similar negative relationship between blood Pb and markers of growth: height, leg and arm length in Polish children aged 7-15 (8µg/dL mean blood Pb and n=899) (Ignasiak *et al.* 2006); height, weight and head circumference in Children aged 2-12 in Dallas (25µg/dL mean blood Pb and n=764; 2µg/dL mean blood Pb and n=390) (Little *et al.* 2009); height in 7-year olds in Mexico (12µg/dL mean blood Pb; n=602) (Kordas *et al.* 2004); height, head circumference, and chest circumference in children aged 6-9 in Greece (12µg/dL mean blood Pb; n=522) (Kafourou *et al.* 1997); height in boys aged 8-9 in Russia (3µg/dL median blood Pb; n=489) (Hauser *et al.* 2008). None of the studies that support an effect level below 5µg/dL control for parental height, which may relate to the cross-sectional nature of the studies. However, parental height is considered in prospective studies as well as a few cross-sectional analyses that support an association with blood Pb levels<10µg/dL (Greene and Ernhart 1991; Kafourou *et al.* 1997; Sanin *et al.* 2001; Schell *et al.* 2009; Shukla *et al.* 1989; Shukla *et al.* 1991; Vivoli *et al.* 1993).

There are also a number of smaller cross-sectional studies with sample size ranges from 330 to 21 that support a negative association between height and concurrent blood Pb levels (**Table 8.4** and Postnatal Growth section of Repro Appendix). The data are not completely consistent, as Zailina *et al.* (2008) did not find a correlation between blood Pb in a study of 269 children in Malaysia in the relative height for age and Tomoum *et al.* (2010) did not find a difference between individuals above and below 10µg/dL in a study of 41 12-year old children in Egypt in the mean height as a percentage of median height. In the Yugoslavia Prospective study, blood Pb in 4-year olds was negatively associated with height in Pristina at blood Pb levels <15µg/dL, but blood Pb levels from 20-40µg/dL in Titova-Mitrovica (a Pb smelter town) were not associated with height (Factor-Litvak *et al.* 1999). Although Vivoli *et al.* (1993) reported a negative association between blood Pb and height in girls from 11-13 years or age, 12-year-old girls and 13-year-old boys, the association was not significant for all age groups (i.e., not for all boys combined, 11-year-old boys, 11-year-old girls, 12-year-old boys, or 13-year-old girls).

There are few studies of growth that include exposure metrics other than blood Pb data. Kim *et al.* (1995) reported a negative association between dentin Pb levels in teeth lost before age 7 to BMI at age 20; however dentin Pb at age 7 or concurrent bone at age 20 were not related to height or weight in the 20 year olds. Sanin *et al.* (2001) reported a negative association between maternal bone Pb or infant blood Pb to weight and weight gain through 12 months of age; but the study did not report data on height.

Summary of support for conclusions

Animal data support a decrease in postnatal growth rate associated with prenatal and developmental Pb exposure at high blood Pb levels in some studies i.e.', 40-100µg/dL Sprague Dawley rats'; see (i.e., 40-100µg/dL Sprague Dawley rats; see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The human data include multiple prospective studies and numerous cross-sectional studies. The three available prospective studies support an association between maternal blood Pb and smaller head circumference from 1-4 years of age (Rothenberg *et al.* 1993; Rothenberg *et al.* 1999; Schell *et al.* 2009). The three available prospective studies addressing the potential association between maternal blood Pb and height or weight do not support an association (Lamb *et al.* 2008; Schell *et al.* 2009), although maternal Pb may be a contributing factor along with infant blood Pb levels (Shukla *et al.* 1989). The prospective studies that investigated the relationship between early blood Pb levels in children and measures of growth support a negative relationship. The two Rothenberg *et al.* (1993; 1999) publications from the Mexico City study reported a negative association between blood Pb in infants at 1 year of age and head circumference up to 4 years of age and Greene *et al.* (1991) reported that blood Pb in 1 year old children was related to subsequent head circumference ($p=0.05$), length ($p=0.06$) and weight ($p=0.08$) in children in the Cleveland Pb study. The strong point of the evidence comes from the number of cross-sectional studies, including large samples such as the NHANES datasets that report a negative association between concurrent blood Pb (mean 2 to 15µg/dL) in children from 1 to 16 years of age and height or other indicators of postnatal growth. However, cross-sectional studies have considerable limitations because they only provide concurrent blood Pb measurements and lack data on cumulative Pb or Pb exposure at earlier time points that may be critical for growth. The consistency of effects across studies, among multiple populations in both males and females lends weight to the evidence that decreased growth indicated by reduced height (and to lesser extent other measures) is associated with blood Pb. The conclusion of *sufficient* evidence that blood Pb levels <10µg/dL in children are associated with decreased growth is based on the combination of strong support from the cross-sectional studies and the additional support from the four prospective studies that evaluate blood Pb levels in children on subsequent growth. Although several studies report an association down to blood Pb levels <5µg/dL, these studies do not adequately control for parental height which is a known important predictor for postnatal growth, so there is inadequate data to evaluate an association with blood Pb levels <5µg/dL. Data from the Cincinnati Lead study suggest that growth may depend on a combination of gestational and early childhood exposure such that effects are only observed in children of mothers with elevated blood Pb that subsequently experienced elevated blood Pb levels in early childhood. The conclusion of *limited* evidence that maternal blood Pb<10µg/dL is associated with decreased head circumference in children

up to 4 years of age is based on three studies from two populations. The NTP's conclusions on a negative association between blood Pb and growth are in line with the 2007 ATSDR Toxicological Profile for Lead, although ATSDR does not specifically identify an effect level for growth and the EPA's 2006 AQCD for Lead reviews the animal data in greater detail than the epidemiological data relating to growth.

8.3.3 Sperm

There is *sufficient* evidence that blood Pb ≥15µg/dL is associated with adverse effects on sperm or semen in adult men, and *inadequate* evidence for adverse effects on sperm at blood Pb levels <15µg/dL. Although there is no single measure of adverse effects that is consistently associated with elevated blood Pb, occupational studies report effects that include lower sperm numbers, decreased motility, reduced semen volume, and an increased percentage of abnormal morphology. Decreased sperm count, density, and/or concentration have been reported in multiple retrospective and cross-sectional studies of men with occupational exposure to Pb at mean blood Pb levels from 15-68µg/dL (**Table 8.5** and Sperm section of Appendix E: Reproductive and Developmental Effects). Among men recruited from infertility or IVF clinics, decreased sperm concentrations and increases in the percentage of abnormal sperm are associated with blood Pb levels from 1-15µg/dL in several studies (Chia *et al.* 1992; Meeker *et al.* 2008; Telisman *et al.* 2007). Men recruited from infertility clinics may represent a susceptible subpopulation, and even within this group, the evidence is not consistent as several studies did not find adverse sperm effects at blood Pb levels <15µg/dL (mean 7-10µg/dL) (Mendiola *et al.* 2011; Xu *et al.* 1993). The conclusion of *inadequate* evidence that blood Pb levels <15µg/dL are associated with adverse effects on sperm is based on the limited number of studies with evidence of effects at these lower blood Pb levels, the lack of consistency in the sperm data from men attending infertility or IVF clinics and the uncertainty in using this patient population to extrapolate to other groups. There are few studies of the relationship between sperm and blood Pb in the general population.

Twelve occupational studies report adverse effects on sperm at blood Pb levels of 15-50µg/dL using mean blood Pb levels of occupationally exposed men or blood Pb levels of workers dichotomized by blood Pb levels. Lower sperm counts or concentration are associated with the following blood Pb concentrations: approximately 15µg/dL (presented graphically) in Naha *et al.* (2005); 20µg/dL in De Rosa *et al.* (2003); approximately 25µg/dL (presented graphically) in Telisman *et al.* (2000); 31µg/dL in Mahmoud *et al.* (2005); ≥40µg/dL in Alexander *et al.* (1996b); ≥44µg/dL in Bonde *et al.* (2002); 48µg/dL in Naha *et al.*, (2006), and 50µg/dL in Naha et al., (2007). Sperm motility is reduced at blood Pb levels of: 20µg/dL in De Rosa *et al.* (2003); 41µg/dL blood Pb in Lancranjan *et al.* (1975); 49µg/dL blood Pb in Lerda i (1992); 53µg/dL blood Pb in Kasperczyk *et al.* (2008); and in three studies already listed for decreased sperm count above (15µg/dL , 48µg/dL, and 50µg/dL (Naha *et al.* 2005; Naha and Chowdhury 2006; Naha and Manna 2007)). Five of the studies support effects in men with mean blood Pb levels from 15 to 31µg/dL. Hsu *et al.* (2009) reported a threshold for increased abnormal sperm

Table 8.5: Studies of sperm and semen parameters associated with low level Pb exposure used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Smelter employees, British Columbia		Retrospective	Blood Pb levels (>40µg/dL) were associated with decreased sperm concentration; *not motility or morphology*	Alexander (1996b)
Supporting	Male employees; n=119			
	Male employees; n=81 of 119 original	Retrospective	Blood Pb levels (mean 22.8µg/dL) were associated with decreased sperm count and concentration; *not motility or morphology*	Alexander (1998)
Supporting	Male Pb workers in Europe; n=486	Cross-sectional	Blood Pb (>50µg/dL) was associated with lower sperm count and density, *not volume, density≤20mil./ml, or chromatin* in Pb workers (n=306; mean blood Pb=31µg/dL) and 197 referents (blood Pb=4.4). Authors suggest 44µg/dL blood Pb threshold for sperm conc.	Bonde (2002)
Supporting	Men at clinic in Croatia; n=240	Cross-sectional	Blood Pb (median 4.9µg/dL) was associated with increased % pathologic sperm, wide sperm, round sperm; *not sperm count, density, viability, motility, or other measures* in men at fertility clinic or donors for artificial insemination.	Telisman (2007)
Not Supporting	Men at infertility clinic in China; n=221	Cross-sectional	Blood Pb (mean 8µg/dL) and semen plasma Pb (mean 1.27µg/dL) were *not correlated to sperm density, motility, viability, morph., or semen volume* in men screened for infertility.	Xu (1993)
Supporting	Men at fert. clinic in Michigan; n=219	Cross-sectional	Blood Pb (median 1.5µg/dL) was associated with a greater OR for below reference sperm concentration; *not count, volume, motility, or morphology*) for men at an infertility clinic.	Meeker (2008)
Supporting	Male Pb workers and referents; n= 200	Cross-sectional	Men with Pb exposure (blood Pb ≥41µg/dL; n=100) show reduced sperm motility, semen vol., and increased abnormal morph. relative to technicians (n=50) and referents (n=50).	Lancranjan (1975)
Not Supporting	Men at fertility clinic in Germany; n=190	Case-control	Blood Pb not reported; grouped by sperm conc., motility, and % normal morphology, there were no differences in semen Pb among 172 infertile men and 18 referents.	Jockenhovel (1990)
Not Supporting	Men at fertility clinic in Finland; n=188	Cross-sectional	Blood Pb not reported; *sperm density, motility, and morphology did not differ* by semen Pb above and below 0.2µg/dL.	Saaranen (1987)
Supporting	Male smelter workers in Belgium; n=159	Cross-sectional	Sperm concentration was significantly reduced in Pb workers (blood Pb=31µg/dL; n=68) compared to hospital staff (referents with blood Pb=3.4µg/dL; n=91).	Mahmoud (2005)
Supporting	Male Pb workers in Croatia; n=146	Cross-sectional	Blood Pb levels were associated with decreased sperm count (blood Pb≥~25µg/dL), decreased sperm density, increased abnormal head morphology, and other parameters in Pb workers (mean blood Pb=39µg/dL; n=98) and referents (Pb=11µg/dL; n=51)	Telisman (2000)
Supporting	Male Pb workers in India; n=130	Cross-sectional	Occupational exposure (mean blood Pb=48µg/dL-n=30; 77µg/dL-n=50) was associated with decreased sperm count, density, motility, semen volume, increased abnormal morphology, and other sperm changes compared to referents (mean Pb 14µg/dL; n=50)	Naha (2006)
Supporting	Male battery workers in India; n=120	Cross-sectional	Occupational Pb exposure (blood Pb≥~14 µg/dL; n=80) was associated with decreased sperm count, density, motility, semen volume, increased abnormal morphology, and other sperm changes compared to referents (blood Pb 7µg/dL; n=40)	Naha (2005)
Supporting	Male Pb workers in	Cross-sectional	Blood Pb levels were associated with decreased sperm count in 39 employees of a Pb	Assennato (1986, 1987)

Table 8.5: Studies of sperm and semen parameters associated with low level Pb exposure used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
	Italy; n=120		battery plant (mean Pb 61µg/dL) and 81 workers at a cement plant (mean Pb 18µg/dL)	
Supporting	Male paint workers in India; n=100	Cross-sectional	Occupational Pb exposure (mean blood Pb ≥50µg/dL; n=50) was associated with decreased sperm count, motility, semen volume, and increased abnormal morphology, DNA hyploidy, compared to non-occupationally exposed workers (n=50)	Naha (2007)
Not Supporting	Male battery workers in England; n=97	Cross-sectional	*Sperm count, density, and motility* were not associated with blood (mean Pb 53µg/dL) or semen Pb (mean Pb 9.6µg/dL). Percent of normal sperm was reduced at p=0.06	Robins (1997)
Supporting	Men in IVF clinic in New York; n=96	Cross-sectional	Blood Pb not reported; semen plasma Pb (mean 40µg/dL) was associated with decrease in sperm motility, conc., morph., other sperm measures, and decreased IVF fertilization.	Benoff (2003a)
Supporting	Male battery workers in Taiwan; n=80	Cross-sectional	Blood Pb levels (mean 40µg/dL) were associated with increased % abnormal sperm and sperm head morph., and DNA denaturation; *not sperm count, semen volume or motility*.	Hsu (2009)
Supporting	Male battery workers in Argentina; n=68	Cross-sectional	Male Pb battery workers with blood Pb ≥49µg/dL (n=38) show reduced sperm motility, semen vol., and increased abnormal morphology relative to referents (n=30).	Lerda (1992)
Blood Pb-Not supporting; Semen Pb-Supporting	Men living near a smelter in Mexico; n=68	Cross-sectional	Blood Pb (mean 9µg/dL) was *not associated with sperm parameters*. Sperm Pb (0.05ng /10^6 cells) was associated with decreased sperm conc., morphology, viability, motility; semen Pb (mean 0.2µg/dL) was associated with decreased volume and increased nuclear chromatin condensation.	Hernandez-Ochoa (2005)
Blood Pb-Not supporting; Semen Pb-Supporting	Men at fertility clinic in Spain; n=60	Cross-sectional	*Sperm motility, conc. and morph. did not differ by blood Pb* (mean=9.8µg/dL); for men at infertility clinic (n=30) and referents (n=30); motility was negatively related to semen Pb (3.0µg/dL).	Mendiola (2011)
Supporting	Male metal workers in Poland; n=63	Cross-sectional	The % motile sperm was decreased in workers with high blood Pb (>40µg/dL n=29, mean Pb 53µg/dL) compared to workers with low Pb (<40µg/dL; n=20) or referents (mean 8µg/dL; n=14); *semen volume, sperm count and morphology were not different*.	Kasperczyk (2008)
Not Supporting	Men in China; n=56	Cross-sectional	Blood Pb not reported; semen plasma Pb (mean 0.78µg/dL) was not correlated with sperm count, density, motility, morphology, or viability; semen Pb was associated with 8-OHdG in men (n=56, population characteristics not reported).	Xu (2003)
Equivocal	Men at infertility clinic in Europe; n=47	Cross-sectional	Blood Pb not reported; authors state negative correlation between Pb and flagellum ball; authors state correlation not detected between pathological changes and elements	Slivkova (2009)
Supporting	Men in andrology clinic in China; n=35	Cross-sectional	Blood Pb was elevated (mean 7.2µg/dL) in men with <40% sperm motility compared to men with >40% sperm motility (mean Pb=5.1µg/dL)	Chia (1992)
Not Supporting	Men in Germany; n=22	Cross-sectional	Blood Pb not reported; semen (0.98µg/dL) and semen plasma (0.77µg/dL) Pb were not correlated to sperm density, count, motility, morph. in men with no occupational Pb.	Noack-Fuller (1993)
Not Supporting	Men in Connecticut; n=21	Cross-sectional	No correlation between semen Pb (mean 5.9µg/dL) and sperm count, density, or semen protein in med. students and techs.; unclear if blood Pb (13.1µg/dL) to sperm examined.	Plechaty (1977)

Table 8.5: Studies of sperm and semen parameters associated with low level Pb exposure used to develop conclusions				
Relevance to conclusions	**Study Description**	**Study Design**	**Key Reproductive/Developmental Findings**	**Reference**
Supporting	Male Pb workers in India; n=20	Cross-sectional	Men with occupational Pb exposure (blood Pb= 42.5µg/dL; n=10) had lower sperm count, decreased % of motile sperm, and increased % of abnormal sperm than referents (n=10).	Chowdhury (1986)
Supporting	Male battery workers in Netherlands; n=19	Cross-sectional	Decrease in blood Pb (median 42 to 19µg/dL) was associated with improved number of motile sperm and penetration in men undergoing treatment to lower blood Pb.	Viskum (1999)
Equivocal	Men with high Pb and referents; n=19	Cross-sectional	Men with chronic high occupational Pb exposure (mean blood Pb=39µg/dL; n=10) did not differ from referents (Pb=16µg/dL; n=9) in sperm vol., motility or % abnormal; 2 men with highest Pb demonstrated peritubular fibrosis, oligospermia, and Sertoli cell vacuolization.	Braunstein (1978)
Supporting	Male semen donors in New York; n=15	Cross-sectional	Blood Pb not reported; semen plasma Pb was associated with decreased sperm motility, premature acrosome loss, and fertilization rate in IVF; not sperm conc.	Benoff (2003b)
Supporting	Men with Pb toxicity in Connecticut; n=7	Case-series	Two of 7 men with occupational Pb intoxication had no sperm; 2 had reduced sperm count; 4 of the 5 with sperm had reduced sperm motility.	Cullen (1984)
Supporting	A firearms instructor in New York	Case Report	A case report of increases in sperm density, total sperm count, and decreases in abnormal morphology in parallel with decreasing blood Pb with chelation therapy in a 41-year old.	Fisher-Fischbein (1987)

* Epidemiological studies of Pb exposure and sperm effects listed by decreasing cohort size and grouped together for overlapping or shared study populations

morphology at blood Pb levels ≥45 µg/dL in Pb battery workers, relative to workers with blood Pb<25µg/dL. However, the extent of DNA denaturation per cell was significantly increased at lower blood Pb levels – both the mid-Pb (25-45µg/dL) and high-Pb (>45µg/dL) workers (Hsu *et al.* 2009). A similar threshold of approximately 25µg/dL was associated with decreased sperm count relative to workers with blood Pb<10µg/dL in a study of 146 industrial workers in which Pb was dichotomized into six groups with n=25 per group and mean blood Pb levels of <10, 12, 25, 35, 42, and 58µg/dL (estimated from Figure 4 in Telisman *et al.* (2000)). Naha *et al.* (2005) reported effects on sperm in Pb-workers with mean blood Pb of approximately 15µg/dL (data from n=10 per group presented graphically) compared to referents with mean blood Pb of 7µg/dL. The Pb workers in Naha *et al.* (2005) have the lowest mean blood Pb level (15µg/dL compared to 48-50µg/dL) in a series of papers from the same group (Naha *et al.* 2005; Naha and Chowdhury 2006; Naha and Manna 2007) that consistently report effects on sperm count, motility, semen volume, and abnormal morphology in all Pb-exposed groups working in paint and battery factories relative to external referents. A cross-sectional study of 68 Pb smelter workers, reported reduced sperm concentration in the Pb workers with a mean blood Pb of 31µg/dL compared to 91 hospital personnel with mean blood Pb of 3.4µg/dL (Mahmoud *et al.* 2005). De Rosa *et al.* (2003) reported lower sperm motility, viability, penetration, and velocity in 85 tollgate workers with blood Pb levels of 20µg/dL compared to referents with blood Pb of 7µg/dL; an analysis that was also significant by linear regression for sperm count. The Naha *et al.* (2005), Telisman *et al.* (2000), Mahmoud *et al.* (2005), De Rosa *et al.* (2003) and Hsu *et al.* (2009) occupational studies report adverse sperm effects down to blood Pb levels of 15-31µg/dL.

A closer examination of the reference populations in the above studies adds to evidence for a threshold closer to 20µg/dL for adverse effects on sperm than a threshold of 40µg/dL; however the data are not consistent. Of the seven studies that report the higher threshold, five rely on internal reference groups with blood Pb levels >10µg/dL (Alexander *et al.* 1996b; Lerda 1992; Naha and Chowdhury 2006; Naha and Manna 2007) or fail to report blood Pb in the referents (Lancranjan *et al.* 1975), so the ability of these studies to detect effects in the lower concentration range is limited. Consideration of the six studies with a reference group <10µg/dL, four of the studies reported effects on sperm at mean blood Pb levels from 15-31µg/dL: at mean blood level of ~15µg/dL in Naha *et al.* (2005), 20µg/dL in De Rosa *et al.* (2003), ~25µg/dL in Telisman *et al.* (2000), and 31µg/dL in Mahmoud *et al.* (2005). The other two studies (Bonde *et al.* 2002; Kasperczyk *et al.* 2008) report effects in men with blood Pb levels >40µg/dL and not in men with blood Pb<40µg/dL. While adjustment for potential confounders is not described in several of the studies, the Telisman *et al.* (2000), Mahmoud *et al.* (2005), and Bonde (2002) studies are all adjusted for factors known to effect sperm count or function such as age and period of abstinence so a lack of adjustment does not explain the difference in the effect levels identified in the studies.

In addition to the occupational Pb exposure studies, there are a number of studies of individuals attending infertility or IVF clinics. Three of these studies reported an association between blood Pb levels below 10µg/dL and several sperm parameters, whereas two studies with similar blood Pb levels did not. In a cross-sectional study of 240 Croatian men that

combined men at an infertility clinic with men donating for artificial insemination, blood Pb in the range of 1.1-14.9µg/dL was associated with increasing percentage of pathologic sperm and wide sperm; with no effect on motility, viability, count, or other measures (Telisman *et al.* 2007). In a study of 219 men attending infertility clinics in Michigan, blood Pb was marginally (p-trend=0.07) related to sperm concentration below reference (<20mil./ml) (Meeker *et al.* 2008). The odds ratio compared to reference sperm concentration was significant for individuals in the 2nd and 3rd quartile relative to the 1st quartile: OR=0.89(95%CI:0.27, 2.89) for the 25-50th percentile (1.1-1.5µg/dL), OR=3.94(95% CI:1.15,13.6) for the 50-75th percentile (1.5-2.0µg/dL), and OR=2.48(95%CI: 0.59, 10.4) for 75th percentile (>2.0µg/dL)(Meeker *et al.* 2008). Chia *et al.* (1992) reported significantly elevated blood Pb (7.2(SD: 6.2)µg/dL vs. 5.1(SD: 2.4)µg/dL; p=0.0034) in men with <40% sperm motility among 35 men attending an andrology clinic in Singapore. The other two studies that report blood Pb and sperm parameters for men attending infertility clinics in China (n=221 at mean blood Pb of 8µg/dL) and Spain (n=60 at mean blood Pb of 10µg/dL) did not find an association between blood Pb and effects on sperm (Mendiola *et al.* 2011; Xu *et al.* 1993); however Mendiola (2011) reported an association between semen Pb levels and increased percentage of immotile sperm.

There are few studies of sperm effects associated with blood Pb levels in the general population that were not patients at infertility clinics, and the available studies do not support an effect of blood Pb on sperm. Hernandez-Ochoa *et al.* (2005) and Plechaty *et al.* (1977) did not detect a significant association between blood Pb (means 9 and 13µg/dL) and sperm parameters, but the studies are relatively small with fewer than 89 men sampled from the two studies combined. It is also worth noting that two Pb-treatment studies support the negative association between high blood Pb levels and sperm parameters. Motility, penetration, and morphology were all improved in 19 Danish Pb-workers treated for high Pb levels in association with lowering blood Pb from a median of 42 to 19.9µg/dL (Viskum *et al.* 1999). Similar results observed in a case-report of a firearms instructor with blood Pb levels that were reduced from 88 to 30µg/dL (Fisher-Fischbein *et al.* 1987).

In studies that report semen Pb levels, the results are inconsistent on whether semen Pb levels are associated with sperm parameters or whether semen Pb is a better exposure metric for effects on sperm. Five reported sperm effects associated with semen Pb (Benoff *et al.* 2003a; Benoff *et al.* 2003b; Hernandez-Ochoa *et al.* 2005; Mendiola *et al.* 2011; Slivkova *et al.* 2009) and six did not find an association (Jockenhovel *et al.* 1990; Noack-Fuller *et al.* 1993; Plechaty *et al.* 1977; Robins *et al.* 1997; Saaranen *et al.* 1987; Xu *et al.* 2003). Several studies (e.g., Benoff *et al.* 2003a; Benoff *et al.* 2003b; Jockenhovel *et al.* 1990; Noack-Fuller *et al.* 1993; Saaranen *et al.* 1987; Slivkova *et al.* 2009; Xu *et al.* 2003) only report semen or sperm Pb, so the utility of semen Pb cannot be compared to blood or bone Pb as a potential biomarker of Pb exposure related to sperm parameters. In studies that report both blood and semen Pb there is some support that both exposure metrics are equally good indicators of Pb exposure as several studies report sperm effects associated with both blood and semen Pb (Naha *et al.* 2005; Naha and Chowdhury 2006; Naha and Manna 2007; Telisman *et al.* 2000) or a lack of an association that is consistent with both blood and semen Pb (Robins *et al.* 1997; Xu *et al.* 1993). Other studies have reported a significant association of sperm parameters with semen Pb (and not

blood Pb) (Hernandez-Ochoa *et al.* 2005; Mendiola *et al.* 2011) or blood Pb (and not semen Pb) (Assennato *et al.* 1986; Kasperczyk *et al.* 2008). In a study of 81 employees of the Cominco smelter, semen and blood Pb levels were associated with decreased sperm concentration; however, after adjustment for ejaculate volume, blood Pb remained significant and semen Pb levels were no longer significantly related to sperm concentration (Alexander *et al.* 1998).

Summary of support for conclusions

Animal data support adverse effects of Pb exposure on sperm and semen including decreased sperm count, reduced sperm motility, and increased morphological abnormalities in sperm in some studies (see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). Although the animal data generally support adverse effects of Pb on sperm, effects may be associated with high doses (40-75µg/dL in rats) and the animal data display differences in sensitivity to sperm effects by species and strain (effects observed down to 16-24µg/dL in rabbits), differences that are likely to be exacerbated by variation in route and duration of exposure, age at initial exposure, and chemical form of Pb used in the experiment. The human data include twelve studies of men with occupational exposure that report effects on sperm or semen at blood Pb levels from 15-50µg/dL. This is supported by six studies reporting effects on time to pregnancy or fertility at similar Pb levels (10-46µg/dL) in men described in **Section 8.3.4 Fertility / Delayed Conception Time** below. The conclusion that there is *sufficient* evidence that blood Pb levels ≥15µg/dL are associated with adverse effects on sperm or semen is based on these studies and specifically the five studies that report effects on sperm at blood Pb levels from 15-31µg/dL. Although occupational studies support adverse sperm effects down to 15µg/dL, the lower threshold of blood Pb level associated with these effects is unclear.

Several studies of men recruited from IVF or infertility clinics report effects at blood Pb levels <10µg/dL. However, men recruited from infertility clinics may represent a susceptible subpopulation, and even within this group, the evidence is not consistent. One challenge in determining the lower limit is that much of the data are from occupational studies in which the mean blood Pb level is 30-40µg/dL. Also, as discussed above, there are few studies of effects in the general population and many studies have a low ability to detect effects associated with lower blood Pb levels as occupational studies generally do not include many men with lower blood Pb levels (i.e., blood Pb <10µg/dL). A number of older studies of sperm or semen parameters (e.g., Lancranjan *et al.* 1975; Lerda 1992) do not adjust for confounding factors such as period of abstinence, age, and smoking. In addition, no human data were located that examine effects of early or developmental exposure on sperm parameters as adults. The NTP's conclusion that there is *sufficient* evidence that blood Pb levels ≥15µg/dL are associated with adverse effects on sperm or semen extends the conclusions of the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) down to 15µg/dL blood Pb from the range of 30-45µg/dL suggested by the 2006 EPA and 2007 ATSDR documents.

8.3.4 Fertility / Delayed Conception Time

There is *sufficient* evidence that paternal blood Pb levels ≥20µg/dL are associated with delayed conception time and *limited* evidence that blood Pb levels ≥10µg/dL in men are associated with

other measures of reduced fertility. Four studies reported increased time to pregnancy or decreased odds of conception over a given time (fecundability) in men with blood Pb levels from 20-40µg/dL, and a fifth study reported decreased odds ratio for probability of live birth in Pb workers with mean blood Pb of 46µg/dL (Fertility /Delayed Conception Time of Appendix E: Reproductive and Developmental Effects). A lower effect level of 10µg/dL is supported by a single large (n=4146) retrospective occupational study that reported increased odds of infertility among men with blood Pb≥10µg/dL (Sallmen *et al.* 2000b); providing *limited* evidence for effects on fertility at blood Pb levels down to 10µg/dL in men. The database of studies that examined male Pb exposure and fertility also includes several occupational studies that did not observe a significant relationship between blood Pb levels in men and fertility or time to pregnancy. There is *inadequate* evidence that blood Pb levels of 10µg/dL and below in women are associated with decreased fertility or greater time to pregnancy because few studies address these effects, and all of them report that maternal blood Pb levels are not associated with time to pregnancy. There is one prospective study of time to pregnancy for women in the general population with blood Pb levels <10µg/dL, and this study found no effect on time to pregnancy. There are several studies of couples attending IVF or infertility clinics that report an association between blood Pb levels from 1-30µg/dL and a decrease in some measure of fertility (e.g., fertilization or embryo quality); additional studies are required to confirm this relationship. Women and men recruited from infertility or IVF clinics may represent a susceptible subpopulation and the effects observed in this patient population should be extrapolated with caution. There are not enough studies of fertility with Pb exposure data for women in the general population or even with occupational exposure to evaluate the potential relationship between Pb exposure and fertility in women.

Six occupational studies in five populations report decreased fertility or greater time to pregnancy in men at blood Pb levels of 10-46µg/dL, and 4 other studies report no association at similar blood Pb levels. In a cross-sectional study that compared 85 tollgate workers with blood Pb levels of 20µg/dL to 85 referents with blood Pb of 7µg/dL De Rosa *et al.* (2003) reported a significant increase from 8 to 15 months in time to pregnancy. Male workers (n=133) in a Pb battery plant in Taiwan had delayed time to pregnancy and reduced FR at blood Pb levels ≥30µg/dL—FR=0.52(95%CI:0.35, 0.77) for blood Pb 30-39µg/dL and FR=0.40(95%CI:0.27, 0.59) for blood Pb ≥40µg/dL (Shiau *et al.* 2004). In retrospective occupational exposure studies of men monitored for Pb exposure by the Finnish Institute of Occupational Health, Sallmen *et al.* (2000a) reported the decreased odds of conception over a given time compared to referents (Fecundability Ratio – FR= 0.57(95%CI:0.34-0.91)) among men (n=502) with blood Pb ≥31µg/dL in analyses restricted to full-term pregnancies. Apostoli *et al.* (2000) reported a significant delay in time to pregnancy for men with blood Pb ≥40µg/dL (p=0.012) in a study of 251 men working at a Pb-related factory in Italy; however, the time to pregnancy was not reduced at lower blood Pb levels and FR analysis suggest a shorter time to pregnancy at lower blood Pb levels. In a cross-sectional study of 365 male Pb battery plant workers with mean blood Pb of 46µg/dL, the odds ratio for probability of live birth was decreased relative to referents with mean blood Pb of 10µg/dL (OR=0.65(95%CI:0.43,0.98)) or relative to pre-exposure (OR=0.43(95%CI:0.25,0.73)) (Gennart *et al.* 1992b). The five studies described above support greater time to pregnancy or reduced fertility in men at blood Pb levels of 20µg/dL, 30µg/dL,

31µg/dL, 40µg/dL, and 46 µg/dL, and a large retrospective study reported increased odds ratio of infertility at even lower blood Pb levels, down to 10µg/dL. In a retrospective studies of men monitored for Pb exposure by the Finnish Institute of Occupational Health, Sallmen *et al.* (2000b) reported increased odds of infertility (RR=1.27(95%CI:1.08-1.51)) and decreased success ratio (SR=0.86(95%CI:0.77,0.97) for pregnancy among wives of male workers (n=4146) with blood Pb≥10µg/dL. Male Pb workers reporting to the New York State Heavy Metals Registry with more than 5 years of Pb work had a reduced fertility rate relative to bus drivers or Pb workers with <5 years of occupational exposure; however blood Pb levels alone were not related to fertility (Lin *et al.* 1996). There are also four retrospective studies that report no association between time to pregnancy or odds ratio for fertility in men occupational exposed to Pb. Paternal Pb was not associated with standardized fertility ratio in 376 male Pb battery workers compared to pre-employment group or workers with blood Pb <25µg/dL (Selevan *et al.* 1984). Odds ratio for reduced fertility did not differ between 1349 Danish Pb workers with a mean blood Pb of 36µg/dL compared to 9596 referents (without blood Pb data) (Bonde and Kolstad 1997). The odds ratio for infertility did not differ between 229 Pb workers dichotomized by blood Pb into 3 groups of <40, 40-60, and >60µg/dL and 125 reference employees classified as non-exposed (Coste *et al.* 1991). Time to pregnancy was not increased in 638 Pb-exposed male workers (mean Pb 29-37µg/dL) compared to external referents (n=236) or an internal control group (n=230) (Joffe *et al.* 2003).

There are few studies investigating the potential relationship between Pb exposure and fertility or time to pregnancy in women. There is one prospective study of time to pregnancy for women in the general population, and Bloom *et al.* (2011a) reported that blood Pb levels (mean 1.5µg/dL) were not associated with time to pregnancy in a study of 80 women in New York. Sallman *et al.* (1995) did not detect a relationship between odds of conception and maternal occupational blood Pb levels in a retrospective study of 121 women in which exposure was estimated based on work descriptions and limited biological measurements.

There are several fertility studies that report exposure metrics other than blood Pb levels. Three case-control studies of infertile men, and two studies of men undergoing IVF examined semen Pb levels and did not report blood Pb data, so the utility of semen Pb cannot be compared to blood or bone Pb as a potential biomarker of Pb exposure related to sperm parameters. Semen Pb was higher in infertile men in two of the studies (Jockenhovel *et al.* 1990; Saaranen *et al.* 1987); but not in a third (Umeyama *et al.* 1986). Benoff *et al.* (2003a; 2003b) reported a negative correlation between semen plasma Pb and *in vitro* fertilization rate in two studies at IVF/AI clinics in studies that did not include blood Pb values. Of the two studies reported follicular Pb levels, one reported an association with fertility and one did not. In the study of 619 women undergoing IVF in Saudi Arabia reported above, follicular Pb levels were not related to fertilization or pregnancy outcome, although blood Pb was associated with decreased OR for fertilization (Al-Saleh *et al.* 2008a). In a small study (n=9 women) that did not report blood Pb, follicular Pb was significantly higher from IVF patients that did not get pregnant than from women that did get pregnant (Silberstein *et al.* 2006).

There are several studies of couples attending IVF or infertility clinics that report an association between blood Pb levels and a decrease in some measure of fertility (e.g., fertilization or embryo quality). Results from studies of men or women reporting to IVF or infertility clinics should be interpreted with caution because they may represent a sensitive subpopulation. In a study of couples undergoing IVF in California, increased blood Pb levels in the women (n=24 and mean blood Pb =0.83µg/dL) were associated with a decreased OR for embryo cell number (a measure of embryo quality) (OR=0.25 (95%CI:0.07,0.86)), or a 75% reduction in embryo quality for each µg/dL increase in maternal blood Pb (Bloom *et al.* 2011b; Bloom *et al.* 2010). Increased blood Pb levels in the men (n=15 and mean blood Pb =1.5µg/dL) were associated with a decreased OR for embryo cell number (a measure of embryo quality) (OR=0.58 (95%CI:0.37,0.91)), or a 42% reduction in embryo quality for each µg/dL increase in paternal blood Pb. There are only two case-control studies of infertile patients with blood Pb levels: one comparing infertile men to fertile controls and one comparing infertile women to controls. Blood Pb (36.8µg/dL(SD: 12) vs 23.2µg/dL(SD: 5.6)) and semen Pb were higher in infertile men at a fertility clinic than controls in a case-control study that examined Pb and smoking (Kiziler *et al.* 2007). Self-reported Pb exposure did not differ between infertile men recruited from infertility clinics and fertile men from prenatal clinics (Gracia *et al.* 2005). In a case-control study of women recruited at an infertility clinic (n=64) and controls from a postpartum clinic (n=83) in Taiwan, blood Pb levels >2µg/dL were associated with an increased OR for infertility (OR=2.94 (95%CI:1.18,7.34)) (Chang *et al.* 2006). In a study of 619 women undergoing IVF, blood Pb levels were significantly higher in women that failed to achieve fertilization (4.1(SD:3.7)µg/dL) than in women in which the IVF produced fertilized eggs (3.26(SD:2)µg/dL); however, blood Pb was not related to pregnancy outcome (Al-Saleh *et al.* 2008a).

Summary of support for conclusions

Animal data support adverse effects of Pb on fertility in several studies at high blood Pb concentrations (>60µg/dL; see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). Effects in male rodents exposed to Pb prior to mating include increased time-to-birth in mice and decreased pregnancy rate in mice or rats (Gandley *et al.* 1999; Pace *et al.* 2005). Female mice exposed to Pb during pregnancy exhibited smaller litter size in several studies (e.g., Pinon-Lataillade *et al.* 1995). The human data include six studies of men with occupational exposure that report increased time to pregnancy or reduced fertility at blood Pb levels from 10-46µg/dL. The conclusion that there is *sufficient* evidence that blood Pb levels ≥20µg/dL are associated with delayed conception time is based on four studies reporting increased time to pregnancy with blood Pb levels of 20-40µg/dL in men. This is supported by numerous studies reporting adverse effects on sperm at similar Pb levels (15-50µg/dL) in men described earlier. Although some studies do not support an association between paternal blood Pb levels and time to pregnancy or fertility, the database (including the sperm data) provides *sufficient* evidence for delayed conception time at blood Pb levels ≥20µg/dL in men. The conclusion that there is *limited* evidence that blood Pb levels ≥10µg/dL in men are associated with decreased fertility is based on the above data with the addition of a single large retrospective occupational study that reported increased odds of infertility among men with blood Pb levels ≥10µg/dL (Sallmen *et al.* 2000b). In the human data, there are few studies of fertility or time to pregnancy with Pb exposure data in women, and both studies that examine

time to pregnancy reported that maternal blood Pb was not related to time to pregnancy. The conclusion of *inadequate* evidence that blood Pb 10µg/dL and below in women are associated with increased time to pregnancy or reduced fertility is based on the limited number of studies addressing these endpoints, and the lack of a significant association with blood Pb reported in some studies on time to pregnancy. The conclusion of *inadequate* evidence for effects on fertility in women is consistent with the 2007 ATSDR Toxicological Profile for Lead and EPA's 2006 AQCD for Lead; however, for men, the 2007 EPA AQCD for Lead (U.S. EPA 2007) states that epidemiological studies suggest a small association between blood Pb levels >45µg/dL in men and increased time to pregnancy. The NTP conclusion of *sufficient* evidence for effects of blood Pb levels ≥20µg/dL in men on time to pregnancy and *limited* evidence that blood Pb levels ≥10µg/dL are associated with reduced fertility is consistent with the conclusions of an effect of Pb in the 2006 EPA and 2007 ATSDR Lead documents, but the NTP outlines the support for a lower effect level (i.e., 20µg/dL rather than 30-45µg/dL). While adjustment for potential confounders are not described in several of the studies, the Shiau *et al.* (2004), Sallmen *et al.* (2000a), and Apostoli (2000) studies all adjusted for factors known to effect fertility such as maternal age and previous abortion, and all of these studies demonstrated an effect of paternal Pb on time to pregnancy. Therefore, a lack of consideration of confounders does not explain the difference between studies that identified an effect of paternal Pb and studies such as Joffe et al. (2003) that did not observe an association between blood Pb in men and time to pregnancy.

8.3.5 Spontaneous Abortion

There is *limited* evidence that maternal blood Pb <10µg/dL is associated with spontaneous abortion. In an extensive review, Hertz-Picciotto *et al.* (2000) concluded that there is consistent evidence from case series and epidemiologic studies in the 19th and early 20th century that Pb exposure at high levels appears to play a role in spontaneous abortion; but the older studies cited lack blood Pb or other biological monitoring data so it is unclear what blood Pb level the data would support. Although more than 20 studies published since the 1970s address maternal or paternal Pb exposure and spontaneous abortion (see Spontaneous Abortion section of Appendix E: Reproductive and Developmental Effects), many lack biological monitoring data. Four of the five retrospective studies that determine exposure by employment or residence report an association between maternal Pb exposure and spontaneous abortion (Driscoll 1998; Nordstrom *et al.* 1978, 1979; Tang and Zhu 2003). Of the studies with blood Pb data, two studies report an association between maternal Pb levels and spontaneous abortion. One case-control, and one nested-case control study found an effect of maternal blood Pb or plasma Pb <10µg/dL and increased risk of spontaneous abortion (Borja-Aburto *et al.* 1999; Yin *et al.* 2008). A number of studies have reported that there was no association between maternal blood Pb levels above or below 10µg/dL and spontaneous abortion. The conclusion that there is *limited* evidence that maternal blood Pb <10µg/dL is associated with spontaneous abortion is based principally on the Borja-Aburto *et al.* (1999) study with the strength of the prospective nested case-control design and additional supporting evidence provided by the Yin *et al.* (2008) data as well as the occupational studies without blood Pb measurements. There is *limited* evidence that paternal blood Pb >31µg/dL is associated with spontaneous abortion. A positive association between paternal blood

Pb>31µg/dL was reported in one occupational study (Lindbohm *et al.* 1991a; Lindbohm *et al.* 1991b) and two retrospective studies that determine exposure by employment but lack blood Pb data (Al-Hakkak *et al.* 1986; Beckman and Nordstrom 1982). Two other studies have reported no association at similar blood Pb levels (i.e., >25µg/dL Alexander *et al.* 1996a; Selevan *et al.* 1984). The conclusion of *limited* evidence that paternal blood Pb >31 is associated with spontaneous abortion is based mainly on the retrospective nested case-control Lindbohm *et al.* (1991a; 1991b) study with support from the occupational studies without blood Pb measurements.

The principal evidence supporting an association between maternal blood Pb levels and spontaneous abortion relies primarily on the Borja-Aburto *et al.* (1999) prospective nested case-control study of women in Mexico. The study reported evidence for a dose-response (p-trend=0.03) and significant ORs for spontaneous abortion of 2.3, 5.4, and 12.2 with maternal blood Pb during the 1st trimester of 5-9, 10-14 and ≥15µg/dL compared to <5.0µg/dL in the reference group (Borja-Aburto *et al.* 1999). The analysis highlighted the careful matching of the timing of exposure measurements in the 35 cases with controls and was adjusted for a range of potential confounders including age, smoking, alcohol consumption, and physical activity. Four retrospective studies support an association between maternal Pb exposure and spontaneous abortion, however no blood Pb data were included and exposure was determined by employment or residence (Driscoll 1998; Nordstrom *et al.* 1978, 1979; Tang and Zhu 2003). Additional support is provided by a case-control study that reported plasma Pb levels with no blood Pb data; maternal plasma Pb (5.3µg/dL in the 40 case women compared to 4.5µg/dL in the 40 controls) was significantly higher in women with anembryonic pregnancy or pregnancy in which early pregnancy appears normal, but for which an embryo visible by ultrasound never develops (Yin *et al.* 2008). Several studies with mean blood Pb levels from 4 to 16µg/dL reported no association between maternal blood Pb levels and spontaneous abortion. Maternal blood Pb during the 1st trimester was not associated with spontaneous abortion at mean blood Pb levels of 4µg/dL in a recent prospective study of 351 women in Iran (Vigeh *et al.* 2010). A prospective study of women residing in a Pb smelting community reported that maternal blood Pb was not statistically different between cases of spontaneous abortion (11.3µg/dL) and controls (10.8µg/dL) (McMichael *et al.* 1986). Two retrospective studies that compared concurrent blood Pb levels (mean of 6µg/dL in Lamadrid-Figueroa *et al.* (2007) and 16µg/dL in Murphy *et al.* (1990)) to recall of pregnancy outcome, reported that concurrent blood Pb in women was not associated with previous history of spontaneous abortion.

The principal evidence supporting an association between paternal blood Pb levels and spontaneous abortion relies on three studies that report an association with paternal Pb exposure. In a retrospective analyses of blood Pb measurements in men occupationally exposed to Pb restricted to within 1 year of spermatogenesis, paternal blood Pb >31µg/dL was associated with an OR of 3.8(95%CI:1.2, 12) compared to men with blood Pb<21µg/dL (Lindbohm *et al.* 1991b). Two retrospective studies of men with occupational exposure to Pb also reported an association between paternal Pb exposure and spontaneous abortion, however exposure was determined by employment and the studies lack blood Pb data (Al-Hakkak *et al.* 1986; Beckman and Nordstrom 1982). Two additional occupational studies that

include blood Pb levels did not detect an association between spontaneous abortion and paternal blood Pb from 25 to >60µg/dL (Alexander *et al.* 1996a; Selevan *et al.* 1984).

In analyses of other biomarkers of Pb exposure, Figueroa *et al.* (2007) found that women with higher plasma/blood ratio had a greater incidence rate for previous abortion (IRR=1.18; p=0.02 for 1 SD increase); however, when examined individually, neither blood, plasma, tibia, nor patella Pb levels were related to spontaneous abortions. In a case-control study that also reported plasma Pb levels, maternal plasma Pb (5.3µg/dL in the 40 case women compared to 4.5µg/dL in the 40 controls) was significantly higher in women with anembryonic pregnancy (Yin *et al.* 2008); however the study does not report Pb levels in whole blood and plasma to whole-blood ratios vary widely (from 0.27 to 0.70%) so it is unclear how plasma Pb data relates to the blood Pb data described above (Hernandez-Avila *et al.* 1998). Placental Pb was significantly higher in women that had a previous miscarriage (Gundacker *et al.* 2010).

Summary of support for conclusions

Animal data were not located that support an association between Pb and spontaneous abortion; although prenatal exposure to Pb has been associated with decreased litter sizes, pup survival, and increased embryonic resorption at very high blood Pb levels (>200µg/dL in mice and rats; see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). As described above, there are few human studies with blood Pb that evaluate the potential association with spontaneous abortion. The conclusions that there is *limited* evidence that maternal blood Pb <10µg/dL and paternal blood Pb >31µg/dL are associated with spontaneous abortion are based primarily on several key studies (i.e., the Borja-Aburto *et al.* (1999) prospective nested case-control study and Lindbohm *et al.* (1991a) retrospective nested case-control study). Additional support for the association is provided by several studies that determine exposure by occupation or residence rather than blood Pb data. In addition, there are studies with blood Pb data that did not find an association between maternal or paternal blood Pb levels and spontaneous abortion. The inconsistency of the results contributes to the determination of *limited* evidence. Although not all studies considered confounders, a lack of adjustment for confounders does not appear to explain the lack of consistency as studies that are both supportive for Pb effects on spontaneous abortion (e.g., Borja-Aburto *et al.* 1999) and not supportive for effects of Pb (e.g., data from Vigeh *et al.* 2010) included adjustments for maternal age, smoking, and other factors. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) both highlight the lack of consistency of the data on spontaneous abortion; however the Borja-Aburto *et al.* (1999) study is highlighted as a well conducted prospective case-control study supporting a significant relationship between maternal blood Pb and spontaneous abortion.

8.3.6 Stillbirth

There is *inadequate* evidence to evaluate the potential association between blood Pb at any level and incidence of stillbirth. Few studies investigate the potential association between Pb exposure and stillbirth, and only a handful have blood Pb or other biological monitoring data (see stillbirth section of Appendix E: Reproductive and Developmental Effects). Of the studies with blood Pb data, none of the studies support an association between maternal or paternal

blood Pb and stillbirth. For example, a prospective study of women residing in a Pb smelting community reported that maternal blood Pb levels were not significantly different between cases of stillbirth (10.3µg/dL during pregnancy and 7.2µg/dL at delivery) and controls (9.9µg/dL during pregnancy and 10.4µg/dL at delivery) (McMichael *et al.* 1986). In a retrospective study that compared concurrent blood Pb levels (mean of 16µg/dL in the residents in a Pb-smelter community and 5.1µg/dL in referents) to recall of pregnancy outcome, Murphy *et al.* (1990) reported that concurrent blood Pb was not associated with previous history of stillbirth (OR=1.0(95%CI:0.6, 1.5)). There are some examples of Pb associated increase in stillbirth in animal literature at very high doses (e.g., >200µg/dL in Sprague-Dawley rats; see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). The dataset available to evaluate this association is small and includes a single prospective study. The 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and 2006 EPA AQCD for Lead (U.S. EPA 2006) do not have specific conclusions on the potential association between Pb exposure and stillbirth.

8.3.7 Fetal Growth and Lower Birth Weight

There are several measures of reduced prenatal growth or intrauterine growth retardation (IUGR): small for gestational age (babies with birth weight below 10th percentile for a given gestational age), lower birth weight (evaluated as a continuous variable), low birth weight (<2500g after at least 37 weeks of gestation), and low birth weight adjusted for gestation length. For this evaluation, any indication of reduced fetal growth is included below.

There is *sufficient* evidence that maternal blood Pb levels <10µg/dL are associated with reduced fetal growth and lower birth weight. The association between maternal Pb exposure and reduced fetal growth is supported by a number of prospective studies with maternal blood Pb data during pregnancy (Bornschein *et al.* 1989; Dietrich *et al.* 1987; Gundacker *et al.* 2010; Jelliffe-Pawlowski *et al.* 2006), a large retrospective cohort study of over 43000 mother-infant pairs (Zhu *et al.* 2010), and a number of cross sectional studies with maternal or cord blood Pb at delivery (Al-Saleh *et al.* 2008b; Bellinger *et al.* 1991; Chen *et al.* 2006; Neuspiel *et al.* 1994; Odland *et al.* 1999; Osman *et al.* 2000; Srivastava *et al.* 2001; Zentner *et al.* 2006) (see Fetal Growth and Lower Birth Weight section of Appendix E: Reproductive and Developmental Effects). There is also one prospective study (Sowers *et al.* 2002) and several cross-sectional studies that report no association between maternal blood Pb and reduced fetal growth at blood Pb levels <10µg/dL and similar results at higher blood Pb levels. Although the results are not entirely consistent across studies, the supporting evidence outlined above with maternal or cord blood Pb levels <10µg/dL from multiple prospective, retrospective, and cross-sectional studies provides *sufficient* evidence at maternal blood Pb levels <10µg/dL are associated with reduced fetal growth and lower birth weight. The large retrospective study of 43288 mother-infant pairs from the New York State Heavy Metals Registry contributes substantially to the conclusions as the study reported a significant association between maternal blood Pb (mean 2.1µg/dL) and lower birth weight in such a large cohort from a study that included adjustment for multiple potential confounders (Zhu *et al.* 2010). The evidence supporting an effect of maternal Pb exposure on reduced fetal growth is further strengthened by a number of studies from a population in Mexico demonstrating that maternal bone Pb is associated with lower birth weight, birth length, and head circumference. These studies provide limited evidence that

maternal bone Pb levels >15.1µg/g (tibia Pb) are associated with reduced fetal growth. Unfortunately the evidence to evaluate the potential association between maternal bone Pb and low birth weight are restricted to a single population. All five of the studies that include maternal bone Pb measurements report a significant negative association between maternal bone Pb levels and fetal growth; but they are all of women attending one of three hospitals in Mexico City from 1994 to 1995. There is *inadequate* evidence that paternal blood Pb at any level is associated with reduced fetal growth because there are few studies of birth weight or related endpoints that include paternal Pb exposure data and the available studies do not support an association with blood Pb in men.

Most prospective or retrospective studies that evaluated the association between maternal blood Pb levels during pregnancy of 10µg/dL or below with measures of fetal growth found a negative association although one study did not find an effect of blood Pb. Maternal blood Pb (mean 2.5µg/dL) at 34-38 weeks of gestation (n=53) of women at General Hospital in Vienna, was associated with lower birth weight (Gundacker *et al.* 2010). Maternal blood Pb levels (mean 7.5µg/dL) of 861 women from the Cincinnati Pb study at 16-28 weeks (2nd trimester into start of 3rd trimester) of gestation were associated with decreased birth weight (Bornschein *et al.* 1989). This was also supported in a smaller analysis of maternal blood Pb (mean 8.3µg/dL) levels sampled at the first prenatal visit in women (n=185) from the Cincinnati Pb study were negatively correlated with birth weight (Dietrich *et al.* 1987). Maternal blood Pb ≥10µg/dL during pregnancy in women in the California Pb surveillance program (n=262) was associated with a greater odds ratio for small for gestational age (OR=4.2(95%CI:1.3,13.9)); however, the association was not significant when analyzed for low birth weight (OR=3.6(95%CI:0.3,40)) (Jelliffe-Pawlowski *et al.* 2006). Maternal blood Pb (mean 2.1µg/dL) during pregnancy or at birth was associated with lower birth weight in a large retrospective study of 43288 mother-infant pairs from the New York State Heavy Metals Registry (Zhu *et al.* 2010). The large retrospective study of over 43000 mother-infant pairs from Zhu et al. (2010) contributes substantially to the conclusions as the study reported a significant association between a maternal blood Pb level well below 10µg/dL and lower birth weight in very large cohort from a study that included adjustment for multiple potential confounders including maternal age, race, parity, smoking, drug abuse, infant sex, and participation in financial assistance as a measure of socioeconomic status(Zhu *et al.* 2010). Maternal blood Pb (mean 1.1µg/dL) sampled at 12, 20, and 28 weeks of gestation in 705 women in Camden, New Jersey was not associated with low birth weight or small for gestational age (Sowers *et al.* 2002). Other than the clear difference in the sample size for the Zhu *et al.* study (2010), there is no obvious difference in blood Pb levels, or statistical adjustments between the 5 studies that support an effect of maternal blood Pb levels <10µg/dL and the one that does not.

Additional studies that sampled maternal or cord blood Pb at delivery also reported an association between blood Pb levels of 10µg/dL and below and measures of fetal growth. Studies of cohorts with higher mean blood Pb levels (i.e., >10µg/dL) are not described below because the discussion focuses on the evidence at blood Pb levels <10µg/dL. However, as with the prospective studies described above, the results are not consistent across all studies. The principal studies with maternal blood Pb levels at delivery are listed below. The data on cord

blood and blood Pb and higher mean blood Pb levels (i.e., >10µg/dL) are not detailed below because they present similar results, but all studies are included in the fetal growth and lower birth weight section of Appendix E: Reproductive and Developmental Effects. Maternal blood Pb means at delivery of 2 to 13µg/dL were associated with lower birth weight in mother-infant pairs from: a case-control study of 30 IUGR and 24 normal births in India (Srivastava *et al.* 2001); a combined population from Russia and Norway (n=262) (Odland *et al.* 1999); and a Pb surveillance program in Taiwan (n=72 low birth weight infants of 1,611 births)(Chen *et al.* 2006). Maternal blood Pb means at delivery of 6 to 10µg/dL were not associated with birth weight, birth length, or head circumference in mother-infant pairs from: women in Cleveland (n=185) (Ernhart *et al.* 1986); Karachi (n=73) (Rahman and Hakeem 2003); or Mexico City (n=272 to 533) (Cantonwine *et al.* 2010b).

There are few studies of fetal growth that include paternal blood Pb levels, and the available evidence provides little support for an association with blood Pb in men. Paternal occupational exposure estimated by job category was associated with low birth weight and small for gestational age in a study of 742 births in the Baltimore-Washington Infant study (Min *et al.* 1996). In two other studies that classified exposure by paternal job category, paternal occupation Pb exposure was not associated with low birth weight in a study of: members of the printers' unions in Oslo, Norway (n=6,251 births) (Kristensen *et al.* 1993); occupational exposure in Norway with possible paternal Pb exposure (n=35,930 births although maternal Pb exposure was associated with low birth weight) (Irgens *et al.* 1998). Paternal blood Pb (mean 13µg/dL) was not associated with small for gestational age or low birth weight in data from a Pb surveillance program in Taiwan (n=72 low birth weight infants of 1,611 births) (Chen *et al.* 2006). Low birth weight was associated with maternal blood Pb levels in the Chen *et al.* (2006) study and occupational Pb exposure in the Irgens (1998) study. Paternal blood Pb was not associated with birth weight or small for gestational age; however blood Pb levels >25µg/dL for more than 5 years was associated with increased relative risk of low birth weight in a study of workers (n=747) reporting to the New York Heavy Metals Registry compared to a referent group of bus drivers (Lin *et al.* 1998).

There are a number of studies of fetal growth that include exposure metrics other than blood Pb data. Data from several studies suggest that maternal bone Pb may be a better exposure metric of the effect of Pb on fetal growth than blood Pb; however the data are restricted to a single population. In a series of studies of women from one of three hospitals in Mexico City, maternal bone Pb measurements were negatively associated with measures of fetal growth, but not maternal blood Pb levels (Cantonwine *et al.* 2010b; Gonzalez-Cossio *et al.* 1997; Hernandez-Avila *et al.* 2002; Kordas *et al.* 2009). Maternal tibia Pb (mean 9.8µg/g) was negatively associated with birth weight at levels >15.1µg/g (Gonzalez-Cossio *et al.* 1997) and birth length at levels >16.6µg/g (Hernandez-Avila *et al.* 2002). Maternal patellar Pb (mean 14µg/dL) was negatively associated with head circumference at levels >24.7µg/g (Hernandez-Avila *et al.* 2002). In further study the authors reported that the H62D genotype may enhance the adverse effect of Pb (Cantonwine *et al.* 2010b) and folate may decrease the adverse effect of Pb (Kordas *et al.* 2009). In a study of 100 mother–infant pairs in France, maternal and infant hair Pb levels were not associated with small for gestational age (Huel *et al.* 1981).

Several studies have investigated the association between placental Pb levels and fetal growth. Two studies (n=53 and n=79 respectively) reported a negative relationship between placental Pb and birth weight and length and/or head circumference (Gundacker *et al.* 2010; Ward *et al.* 1990). Two case-control studies reported higher placental Pb levels in fetal growth restriction births (n=20) (Llanos and Ronco 2009) or intrauterine growth retardation births (n=50) (Richter *et al.* 1999). Three studies reported a lack of an association between placental Pb and birth weight in studies of: 161 women from the Yugoslavia Prospective Study (Loiacono *et al.* 1992); 262 women constituting a combined population from Russia and Norway (Odland *et al.* 2004); 126 births at the Birmingham Maternal Hospital (Wibberley *et al.* 1977). The determination of utility of placental Pb as an exposure metric rather than blood Pb is difficult to ascertain for low birth weight because most studies do not report exposure data for both measures and the results are inconsistent. In one study that did include both placental Pb and maternal blood Pb levels, low birth weight was associated with maternal blood Pb levels in the Gundacker et al. (2010) as well as placental Pb as described above.

Summary of support for conclusions

Animal data support an association between Pb and lower birth weight at high blood Pb levels (54-300µg/dL in squirrel monkeys, mice, and rats; see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). As described above, there are a number of epidemiological studies that report effects of Pb on fetal growth or birth weight. The conclusion of *sufficient* evidence that maternal blood Pb <10µg/dL is associated with reduced fetal growth is based on 4 prospective studies with maternal blood Pb during pregnancy, a large retrospective cohort study, and a number of cross sectional studies with maternal or cord blood Pb at delivery. Although the results are not entirely consistent across studies and some studies report that prenatal blood Pb levels <10µg/dL are not associated, the supporting studies provide sufficient evidence that maternal blood Pb levels <10µg/dL is associated with reduced fetal growth. In particular, the large retrospective study of 43288 mother-infant pairs from the New York State Heavy Metals Registry contributes substantially to the conclusion of *sufficient* evidence because is based on such a large cohort with a low mean blood Pb level (2.1µg/dL) and the analyses adjust for multiple potential confounders including maternal age, race, parity, smoking, drug abuse, infant sex, and participation in financial assistance as a measure of socioeconomic status (Zhu *et al.* 2010). Additional support is provided by a number of cross-sectional studies with maternal or cord blood Pb <10µg/dL at delivery, and a group of studies from a single population that demonstrate a negative relationship between maternal bone Pb and fetal growth. The conclusion of *inadequate* evidence that paternal blood Pb at any level is associated with fetal growth is based on a small number of studies and general lack of observed effect. The NTP conclusion that there is *sufficient* evidence for effects of maternal blood Pb ≤10µg/dL on fetal growth is stronger than the 2006 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2007 EPA AQCD for Lead (U.S. EPA 2007) in which the evidence was characterized as inconsistent. The NTP conclusion that there is *inadequate* evidence for effects of parental blood Pb on fetal growth is consistent with the 2006 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2007 EPA AQCD for Lead (U.S. EPA 2007).

8.3.8 Preterm Birth and Gestational age

There is *limited* evidence that maternal blood Pb levels <10µg/dL are associated with preterm birth or reduced gestational age due to inconsistent results in studies with low blood Pb levels. Increasing maternal blood Pb levels during pregnancy were associated with preterm birth or reduced gestational age in 2 prospective studies(Cantonwine *et al.* 2010a; Vigeh *et al.* 2011) and 5 cross-sectional studies (Fagher *et al.* 1993; Odland *et al.* 1999; Patel and Prabhu 2009; Satin *et al.* 1991; Torres-Sanchez *et al.* 1999) with exposure data from maternal or cord blood Pb at delivery with mean blood Pb levels <10µg/dL (see Preterm section of Appendix E: Reproductive and Developmental Effects). However, there are a number of cross-sectional studies and several prospective studies (e.g., Bornschein *et al.* 1989; Sowers *et al.* 2002) that report no association between maternal blood Pb and preterm birth at the same blood Pb levels. In addition, the large retrospective study of 43288 mother-infant pairs from the New York State Heavy Metals Registry did not find an association between maternal blood Pb (mean 2.1µg/dL) and preterm birth (Zhu *et al.* 2010). There is *inadequate* evidence that paternal blood Pb at any level is associated with preterm birth due to the small number of studies and general lack of observed effects in studies that report blood Pb levels in men.

Studies that compare maternal blood Pb levels during pregnancy with preterm birth report mixed results; four studies found an association between blood Pb (mean 4-10µg/dL) and preterm birth or decreased gestational age and three did not find an effect of blood Pb (mean 1-23µg/dL). The data are also inconsistent when examined by maternal Pb levels from a specific trimester. Maternal blood levels (mean 6-7µg/dL by trimester) in the 1st and 2nd trimester, but not the 3rd trimester, were negatively associated with gestational age in a prospective study of 327 women in Mexico City (Cantonwine *et al.* 2010a). Maternal blood levels in the 1st trimester was associated with preterm birth and negatively associated with gestational age in a prospective study of 348 women with mean blood of 4µg/dL in Iran (Vigeh *et al.* 2011). Although maternal blood Pb ≥10µg/dL was associated with preterm birth in a study of women in the California Pb surveillance program (n=262), in contrast to the Cantonwine and Vigeh data, the effect was significant during 2nd and 3rd trimester, not the 1st trimester (Jelliffe-Pawlowski *et al.* 2006). Maternal blood Pb (mean 8.3µg/dL) levels sampled at the first prenatal visit in women (n=185) from the Cincinnati Pb study were negatively correlated with gestational age (Dietrich *et al.* 1987). The Dietrich *et al.* (1987) results were reported as part of an analysis of neurological data and the study population was a subset of women evaluated in a later study that did not find a significant association (Bornschein *et al.* 1989). Maternal blood Pb levels (mean 7.5µg/dL) at 16-28 weeks (2nd trimester into start of 3rd trimester) of gestation were not associated with gestational age (Bornschein *et al.* 1989). Maternal blood Pb (mean 1.1µg/dL) sampled at 12 weeks (1st trimester), 20 weeks (2nd trimester), and 28 weeks (3rd trimester) of gestation in 705 women in Camden, New Jersey was not associated with preterm birth (Sowers *et al.* 2002). Maternal blood Pb at mid-pregnancy (2nd trimester, mean=20µg/dL) and delivery (mean =23µg/dL) were not associated with preterm birth in women from the Yugoslavia Prospective study (n=907) (Factor-Litvak *et al.* 1991). Excluding the Dietrich *et al.* (1987) study, there are three prospective or retrospective studies that support an association between maternal blood Pb from 4-10µg/dL during pregnancy and preterm birth (Cantonwine *et al.* 2010a; Jelliffe-Pawlowski *et al.* 2006; Vigeh *et al.* 2011) and

three studies that do not support an association with maternal blood Pb (Bornschein *et al.* 1989; Factor-Litvak *et al.* 1991; Sowers *et al.* 2002). The studies that do not support a relationship with Pb exposure are older, but all of these studies considered and adjusted for potential confounders including maternal age, smoking, and other factors. The studies that do not support a relationship with Pb exposure also have larger sample sizes (n= 705 to 907) than the studies that support an effect of Pb (n=262 to 348).

Additional studies that sampled maternal or cord blood Pb at delivery also reported inconsistent results for these indicators of Pb exposure and preterm birth. Maternal blood Pb or cord blood at delivery at mean Pb levels from 1 to 15µg/dL were associated with preterm birth or reduced gestational age in mother-infant pairs from: five cities in California (n=723) (Satin *et al.* 1991); the Port Pirie birth cohort study (n=721) (McMichael *et al.* 1986); Rolla and Columbia Missouri (n=502) (Fahim *et al.* 1976); Mexico City (n=620) (Torres-Sanchez *et al.* 1999); a combined population from Russia and Norway (n=262) (Odland *et al.* 1999); Glasgow (n=236) (Moore *et al.* 1982); a hospital in India (n=205) (Patel and Prabhu 2009); and a small combined population from Poland and Sweden (gestational age) (n=17 preterm and n=13 controls)(Fagher *et al.* 1993). Maternal blood Pb at delivery or cord blood at mean Pb levels from 2 to 30µg/dL were not associated with preterm delivery or reduced gestational age in mother-infant pairs from: the New York State Heavy Metals Registry (n=43,288) (Zhu *et al.* 2010); the Brigham and Women's Hospital (n=3503) (Bellinger *et al.* 1991); Louisville General Hospital (n=635) (Angell and Lavery 1982); Memphis (n=102) (Jones *et al.* 2010); and New York City (n=100) (Rajegowda *et al.* 1972). The database of studies that determined exposure from maternal or cord blood Pb at delivery includes a number of studies that do not report appropriate adjustments (e.g., Fahim *et al.* 1976), but there are also positive (e.g. , Torres-Sanchez *et al.* 1999) and negative studies (e.g. , Zhu *et al.* 2010) for the effects of Pb that adjusted for potential confounders including maternal age, parity, and smoking.

There are few studies that address the relationship between paternal blood Pb and preterm births and the available data do not support a relationship between blood Pb in men and preterm birth. In a study of over 3000 births to male workers in the New York State Heavy Metals Registry, Lin *et al.* (1998) reported that parental blood Pb >25µg/dL did not affect the relative risk of preterm births (RR=0.89 (95%CI:0.64,1.26)) compared to referents (bus drivers); however continued blood Pb >25µg/dL for more than 5 years was associated with increased relative risk of preterm births (RR=3.03 (95%CI:1.35,6.77)) relative to workers that did not consistently report a blood Pb level greater than 25µg/dL. In a similar worker surveillance program in China, paternal blood Pb (mean 14µg/dL) was not associated with preterm birth (Chen *et al.* 2006). Paternal Pb exposure determined by paternal job category was also not associated with preterm birth in a study of: members of the printers' unions in Oslo, Norway (n=6,251 births) (Kristensen *et al.* 1993); births from the National Natality and Fetal Mortality survey in the United States (Savitz *et al.* 1989). Occupational exposure in Norway with possible paternal Pb exposure (n=35,930 births) was associated with longer-term births, and maternal Pb exposure was associated with preterm birth (Irgens *et al.* 1998).

Several studies also address other exposure metrics such as hair, or placental Pb levels. Huel *et al.* (1981) reported higher hair Pb levels in mothers and offspring from preterm births than normal births. Two studies reported higher placental Pb levels in preterm (or combined analysis of preterm and PROM births) than births with normal delivery (Falcón *et al.* 2003; Ward *et al.* 1990). However, four other cross-sectional studies did not find a significant relationship between placental tissue Pb concentrations and preterm births (Baghurst *et al.* 1991; Fahim *et al.* 1976; Loiacono *et al.* 1992; Ward *et al.* 1987). Cantonwine (2010a) did not find a significant association between maternal plasma Pb and preterm birth, although the relationship was significant with maternal blood Pb levels.

Summary of support for conclusions

Animal data were not located that support an effect of Pb on preterm delivery, although potentially related endpoints such as pup survival and birth weight were adversely affected at high blood Pb levels (54-300µg/dL in squirrel monkeys, mice, and rats; see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). As described above, the human data are not consistent for an effect of Pb on preterm birth. Although a number of prospective studies with maternal blood Pb levels during pregnancy and cross-sectional studies with cord blood Pb levels at delivery reported an association between prenatal blood Pb levels <10µg/dL and preterm birth, the conclusion of *limited* evidence is based on the inconsistent results and because a large retrospective study that not find an association between maternal blood Pb levels and preterm birth. In particular, the large retrospective study of the New York State Heavy Metals Registry included 43288 mother-infant pairs and did not find an association between maternal blood Pb (mean 2.1µg/dL) and preterm birth (Zhu *et al.* 2010). The conclusion of *inadequate* evidence that paternal blood Pb at any level is associated with preterm birth or reduced gestational age is based on a small number of studies and general lack of observed effect. Of the five studies located that address paternal exposure and preterm birth: three report no effect, one reports a Pb-associated increase in gestational age, and one reports an association with persistently elevated paternal Pb (>25µg/dL for 5 years) and preterm birth. The NTP conclusion that there is *limited* evidence for effects of maternal or cord blood Pb ≤10µg/dL on preterm birth is in line with the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2006 EPA AQCD for Lead (U.S. EPA 2007) in which the evidence was characterized as inconsistent.

8.3.9 Endocrine Effects

There is *inadequate* evidence to evaluate the potential association between blood Pb and major endocrine or changes in hormone levels due to inconsistency of effects across available studies (see endocrine section of Appendix E: Reproductive and Developmental Effects). The data are inconsistent for the effects of Pb on LH (luteinizing hormone), FSH (follicle stimulating hormone), T (testosterone), and for other hormones, including E_2 (estradiol-17β), prolactin (PRL), thyroid stimulating hormone (TSH), thyroxin (T_4), triiodothryonine (T_3), parathyroid (PTH), inhibin B, and insulin like growth hormone (IGF-1).

In studies that examined the relationship between blood Pb and LH or FSH, the results are inconsistent. Blood Pb levels (mean 30 to 69µg/dL) were not associated with serum levels of LH

or FSH in a number of studies of men with occupational Pb exposure (Alexander *et al.* 1996b; Assennato *et al.* 1986; Gennart *et al.* 1992a; Hsieh *et al.* 2009; Mahmoud *et al.* 2005; Naha and Manna 2007; Ng *et al.* 1991; Telisman *et al.* 2000) and not at lower blood Pb levels (mean of 3 to 10µg/dL) in men or women recruited at infertility clinics (Chang *et al.* 2006; Meeker *et al.* 2010; Mendiola *et al.* 2011) or men without occupational Pb exposure (Telisman *et al.* 2007). Basal levels of LH and FSH did not differ between Pb workers and referents; however, GnRH-stimulated FSH was decreased in Pb-workers with median blood Pb levels of 31µg/dL(Erfurth *et al.* 2001). In a study of 23 Pb workers serum LH was increased in Pb workers (mean 60-73µg/dL) relative to referents (mean 17µg/dL) (Rodamilans *et al.* 1988). Several studies have reported that FSH was increased (Cullen *et al.* 1984; De Rosa *et al.* 2003; McGregor and Mason 1991; McGregor and Mason 1990) or decreased (Gustafson *et al.* 1989) in men at blood Pb levels of 20-45 µg/dL relative to referents. Results of study by Krieg et al. (2007) of women from NHANES III suggest that the effect of Pb on FSH and LH is modified by reproductive status (e.g., menopausal status) or external hormones. FSH was increased with increasing blood Pb in women from NHANES III (mean blood Pb 2.8µg/dL), but decreased with blood Pb in women taking birth control pills (Krieg 2007). LH was increased with increasing blood Pb in post-menopausal women from NHANES III, and LH was not associated with blood Pb in other women (Krieg 2007).

The data are inconsistent for the effects of Pb on T, thyroid hormones (TSH, T_4, and T_3) and for other hormones, including E_2 and PRL. Blood Pb levels (mean 30 to 69µg/dL) were not associated with serum levels T (testosterone) in a number of studies of men with occupational Pb exposure (Alexander *et al.* 1996b; Assennato *et al.* 1986; Gennart *et al.* 1992a; Hsieh *et al.* 2009; Mahmoud *et al.* 2005; Naha and Manna 2007; Ng *et al.* 1991). Several studies have reported lower basal or stimulated T with occupational exposure in the same blood Pb range (Braunstein *et al.* 1978; Rodamilans *et al.* 1988; Telisman *et al.* 2000). At lower blood Pb levels (<10µg/dL) exposure was associated with increased serum T in a study of 240 men without occupational Pb exposure (Telisman *et al.* 2007) and in 219 men recruited from infertility clinics, but not after adjustment for exposure to other metals (Meeker *et al.* 2010). However, Mendiola et al. (2011) did not find an association between blood Pb (mean 10µg/dL) and serum T in 60 men recruited at an infertility clinic. Occupational Pb exposure in men at mean blood Pb levels 30-40µg/dL were not associated with E_2 in one study (Mahmoud *et al.* 2005), but was associated with decreased E_2 in another study (Telisman *et al.* 2000). AT blood Pb levels <10 µg/dL, Pb was associated with increased E_2 in a case-control study of women recruited at an infertility clinic (n=64) and controls from a postpartum clinic (n=83) in Taiwan (Chang *et al.* 2006). Also, at blood Pb levels <10µg/dL, E_2 was increased and PRL was decreased in association with Pb in men without occupational Pb exposure (Telisman *et al.* 2007). In men with occupational exposure to Pb and mean Pb levels from 30 to 60 µg/dL, blood Pb was not associated with PRL or did not differ from referents (Assennato *et al.* 1986; Ng *et al.* 1991; Roses *et al.* 1989; Telisman *et al.* 2000). Serum TSH was elevated in Pb gas station workers with blood Pb mean of 51µg/dL relative to referents (Singh *et al.* 2000). In contrast, blood Pb levels were negatively related to TSH in women, but not in men among people in Quebec that regularly eat freshwater fish men; T_3 and T_4 were not associated with Pb levels <10µg/dL (Abdelouahab *et al.* 2008). Serum levels of TSH, T_3 or T_4 did not differ between men with high

occupational Pb (mean 51µg/dL) to referents (21µg/dL) (Gennart *et al.* 1992a), male Pb workers with mean blood Pb of 31µg/dL (Erfurth *et al.* 2001), male Pb workers with mean blood Pb of 24µg/dL (Schumacher *et al.* 1998); in a study of 24 newborns with mean blood Pb levels of 6µg/dL (Iijima *et al.* 2007), or 68 children <8 years of age with range of blood Pb from 2-77µg/dL (Siegel *et al.* 1989). Two studies of men with occupational Pb exposure (mean Pb level 42 to 51µg/dL) reported opposite results: T_4 and free T_4 were higher in 75 Pb workers than 68 matched referents (Lopez *et al.* 2000); T_4 and free T_4 were negatively associated with blood Pb in a study of 54 workers at a brass foundry (Robins *et al.* 1983). In 309 mother-children pairs from the Yugoslavia Prospective study, maternal T_4 was inversely associated with blood Pb in women from a Pb smelting town (median blood Pb 20µg/dL), but not in a reference town (median blood Pb 6µg/dL) (Lamb *et al.* 2008). Cumulative Pb exposure was associated with increased serum inhibin B levels in several studies of male Pb workers: n=181 (Hsieh *et al.* 2009); n=68 (Mahmoud *et al.* 2005).

There are few studies of Pb and hormone levels in children. Two available studies suggest that Pb may decrease LH and FSH. In a study of 41 children aged 10-13 in Egypt, boys and girls with blood Pb >10µg/dL had lower FSH and LH; boys had lower serum T, but there was no effect of Pb on E_2 (Tomoum *et al.* 2010). Similarly, Vivoli et al. (1993) reported decreased LH and FSH in boys with blood Pb≥10µg/dL in a study of 418 children in Italy; T and E_2 were not related to blood Pb levels. Girls aged 6-11 in NHANES III blood Pb ≥1 µg/dL had lower levels of inhibin B (Gollenberg *et al.* 2010).

Summary of support for conclusions

Animal data support an association between Pb exposure and altered hormones, particularly decreased LH, FSH, and E_2 in females at high blood Pb levels (30-300µg/dL in Cynomolgus monkeys and rats; see ATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). Rodent data also support an effect of Pb testosterone in some studies; however, even within the animal literature, increase, decrease, and lack of effect are reported. Animal data also support decreased IGF-1, LH, and E_2 as potential mechanisms for the Pb-associated delay in puberty. Animal data also support an effect of developmental Pb exposure on the hypothalamic-pituitary-adrenal axis and changes in the stress response or basal corticosteroids. The human data on potential associations between Pb and LH, FSH, and T are inconsistent and for other hormones the data are inconsistent as well as limited in nature. Therefore, the NTP determined there was *inadequate* evidence to conclude that blood Pb was associated with specific hormone changes. The conclusion of *inadequate* evidence for endocrine effects of Pb in humans is in line with the discussion of the general inconsistencies in the human database with the ATSDR and EPA; however, the 2006 EPA AQCD for Lead (U.S. EPA 2007) states that the toxicological data (animal data) support Pb as an endocrine disruptor in males and females at various points along the hypothalamic-pituitary-gonadal axis. Additional data, particularly data from prospective studies with appropriate consideration and control for timing in the reproductive cycle, age, and other factors, are required to clarify the potential relationship between blood Pb and endocrine parameters.

8.3.10 Congenital Malformations

There is *inadequate* evidence to evaluate the potential association between blood Pb and congenital malformations. There are few studies that investigate the potential association between Pb exposure and malformations, and only a handful have blood Pb or other biological monitoring data (see Congenital Malformations section of Appendix E: Reproductive and Developmental Effects). Of the studies with blood Pb data, only one supports an association between blood Pb levels. In a retrospective analysis, Needleman *et al.* (1984) found the relative risk of minor congenital anomaly to be positively related to cord blood at all blood Pb levels [6.3µg/dL RR=1.87 9 (1.4, 2.4); 15µg/dL RR=2.39 (1.7,3.4); 24µg/dL RR=2.73 (1.8, 4.2)]. There are few other published studies and most lack blood Pb data or other biomarkers and exposure is based on job categories or indirect measures. For example residence in the ceramic district in Italy, an area known for higher Pb exposure levels, was associated with increased relative risk of malformations (RR=1.48(95%CI:1.15,1.89)) including elevated risk of hydrocephalus, oral clefts, cleft lip, and malformations of the ear, heart, cardiovascular system, musculeoskelatal system, and integument (Vinceti *et al.* 2001).

Several occupational studies investigated the relationship between paternal blood Pb and congenital malformations, and the available evidence is inconsistent. Blood Pb levels determined from monitoring data for 929 employees of the Cominco smelter were not associated with odds ratio for a combined analysis of still births and birth defects (Alexander *et al.* 1996a). Paternal occupational Pb exposure was not associated with congenital malformations in a study of 764 workers at a copper smelter that compared rates of malformations between pregnancies following employment to pregnancies that took place prior to occupational Pb exposure (Beckman and Nordstrom 1982). Estimated paternal blood Pb levels based on job categories were associated with an increased odds ratio for congenital malformations (OR=3.2(95%CI:1.0,10.2) when evaluated along with paternal smoking (Sallmen *et al.* 1992).

A number of studies have examined the potential association between Pb exposure and neural tube defects. The evidence that Pb exposure is associated with neural tube defects is inconsistent, and both studies that include blood Pb data do not support an association with Pb exposure. Two recent case-control studies did not find an association between incidence of neural tube defects and maternal or cord blood Pb at birth in mother-infant pairs in Turkey (Zeyrek *et al.* 2009) as well as maternal blood Pb taken 5-6 weeks after birth in Mexican-Americans living near Texas-Mexico border (Brender *et al.* 2002; 2006). Dawson et al. (1999) reported higher Pb levels in amniotic fluid of NTD cases (n=11) than in controls (n=29). In a study that looked for serious birth defects among births in Norway with possible parental occupational Pb exposure by job classification, Irgens *et al.* (1998) reported that maternal Pb exposure (of n=1803 exposed births) was associated with a greater odds ratio for neural tube defects OR=2.87(95%CL:1.05,6.38); but not paternal exposure (of n=35930 exposed births). Bound *et al.* (1997) reported a significant association between risk of neural tube defects in a case-control study that determined Pb exposure by residence in a district in the United Kingdom known to have higher drinking water levels of Pb. Drinking water levels of Pb or residence near a hazardous waste site with known Pb were not associated with neural tube

defects or anencephalus in several case-control studies (Croen *et al.* 1997; Elwood and Coldman 1981; Macdonell *et al.* 2000).

Several studies have examined the potential association between Pb exposure and cardiovascular defects. The evidence that Pb exposure is associated with cardiovascular defects is inconsistent, from few studies, and lacks good exposure data. The study of residents in the Pb-associated ceramic district in Italy described above, reported increased relative risk of prevalence of specific heart malformations (RR=2.47(95%CI:1.57,3.70)) and general cardiovascular malformations (RR=2.59(95%CI:1.68,3.82)) (Vinceti *et al.* 2001). Drinking water levels of Pb or residence near a hazardous waste site with known Pb were not associated with congenital heart disease or cardiovascular anomalies in several case-control studies (Aschengrau *et al.* 1993; Croen *et al.* 1997; Zierler *et al.* 1988). A case-control study of 54 children with total anomalous pulmonary venus return and 522 matched controls in the Baltimore-Washington Infant study reported a significant odds ratio for paternal Pb exposure OR=1.83(95%CI:1.00,3.42) (Jackson *et al.* 2004).

Several studies have also examined the potential association between Pb exposure and cleft lip or cleft palate. The evidence that Pb exposure is associated with oral clefts is from few studies, all of which lack biological exposure data. The study of residents in the Pb-associated ceramic district in Italy described above, reported increased relative risk of oral clefts (RR=2.28(95%CI:1.16,4.07)) and cleft lip RR=2.43(95%CI:1.13,4.62)) (Vinceti *et al.* 2001). A case-control study of 100 mothers of babies with oral clefts and 751 controls reported an increased odds ratio of oral clefts (OR=4.0(95%CI:1.3,12.2)) (Lorente *et al.* 2000). Paternal Pb exposure by job category among 6251 births to male members of the printer's union in Norway was associated with an increased standardized morbidity ratio for cleft lip in boys (SMR=4.1(95%CI:1.8,8.1)) (Kristensen *et al.* 1993).

Summary of support for conclusions

Limited animal data support an effect of Pb on congenital malformations, principally tail defects and general external malformations in NOS rats, although increased fetal mortality was reported in some studies at high blood Pb levels (54-300μg/dL in squirrel monkeys and mice; seeATSDR 2007; U.S. EPA 2006 for recent reviews of the animal data). In the human data, there are few studies of congenital malformations with parental Pb exposure data. Although studies have reported general effects on congenital malformations, and specific effects on neural tube defects, cardiovascular defects, and oral clefts, the results are inconsistent and the studies generally lack biological exposure data. The NTP determination that there is *inadequate* evidence to conclude parental blood Pb levels are associated with congenital malformations is consistent with the 2007 ATSDR Toxicological Profile for Lead (ATSDR 2007) and the 2006 EPA AQCD for Lead (U.S. EPA 2007).

8.4 Conclusions

The NTP concludes there is *sufficient* evidence that blood Pb levels <10 μg/dL are associated with adverse health effects on development in children and reproduction in adult women (see

Table 8.6 for complete list of reproductive and developmental effects conclusions). In some studies blood Pb levels at and below 2µg/dL are associated with adverse effects (e.g., Denham *et al.* 2005 for delayed onset of puberty; Wu *et al.* 2003) and the ability to discriminate effects at the lower dose may depend on the availability of a reference population with lower blood Pb levels or the precision of blood Pb measurements. In children, there is *sufficient* evidence that blood Pb levels <10µg/dL are associated with delayed puberty and decreased postnatal growth and *limited* evidence that delayed puberty is associated with blood Pb levels <5µg/dL. In adults, there is *sufficient* evidence that maternal blood Pb levels <10µg/dL are associated with reduced fetal growth and limited evidence that maternal blood Pb levels <10µg/dL are associated with spontaneous abortion and preterm birth. In men there is *sufficient* evidence that blood Pb levels ≥15µg/dL are associated with adverse effects on sperm or semen and blood Pb levels ≥20 are associated with delayed conception time. There is *limited* evidence that blood Pb levels ≥10µg/dL in men are associated with other measures of reduced fertility and that blood Pb >31µg/dL are associated with spontaneous abortion.

Table 8.6: NTP conclusions on reproductive and developmental effects of low level Pb

Health Effect	Population Or Exposure window	NTP Conclusion	Blood Pb Evidence	Bone Pb Evidence
Delayed Puberty	Prenatal	*Inadequate*	No data	No data
	Children	*Sufficient*	Yes, <10µg/dL	No data
		Limited	Yes, <5µg/dL	
Postnatal Growth	Prenatal	*Limited*	Yes, <10µg/dL	one study
	Children	*Sufficient*	Yes, <10µg/dL	one study available, no evidence of an association
Sperm parameters	Children	*Inadequate*	No data	No data
	Men	*Sufficient*	Yes, ≥15µg/dL	No data
Fertility / Delayed conception time	Men –time to conception	*Sufficient*	Yes, ≥20µg/dL	No data
	Men - fertility	*Limited*	Yes, ≥10µg/dL (one study)	No data
	Women	*Inadequate*	Unclear	No data
Spontaneous Abortion	Men	*Limited*	Yes, >31µg/dL	No data
	Women	*Limited*	Yes, <10µg/dL	No data
Stillbirth	Adults	*Inadequate*	Unclear	No data
Reduced Fetal Growth and Lower Birth Weight	Men	*Inadequate*	Unclear	No data
	Women	*Sufficient*	Yes, <10µg/dL	Yes, tibia
Preterm Birth and Gestational Age	Men	*Inadequate*	Unclear	No data
	Women	*Limited*	Yes, <10µg/dL	No data
Endocrine effects	Adults	*Inadequate*	Unclear	one study
Birth Defects	Adults	*Inadequate*	Unclear	No data

9.0 REFERENCES

9.1 Executive Summary

ABLES (2009). Adult Blood Lead Epidemiology and Surveillance (ABLES) program case definition for an Elevated Blood Lead Level.

ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Barbosa, F., Jr., Tanus-Santos, J. E., Gerlach, R. F., and Parsons, P. J. (2005). A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. *Environ Health Perspect* **113**, 1669-1674.

Barry, P. S. (1981). Concentrations of lead in the tissues of children. *Br J Ind Med* **38**, 61-71.

CDC (2007). Interpreting and managing blood lead levels <10 ug/dl in children and reducing childhood exposures to lead: recommendations of CDC's Advisory Committee on Childhood Lead Poisoning Prevention. In Morbidity and Mortality Weekly Report, Vol. 56, pp. 1-15. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2010a). Elevated Blood Lead Levels: 2010 case definition for the National Notifiable Non-Infectionus Conditions. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2010b). Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women: Recommendations from the Advisory Committee on Childhood Lead Poisoning Prevention. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2011). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, February 2011. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Coon, S., Stark, A., Peterson, E., Gloi, A., Kortsha, G., Pounds, J., Chettle, D., and Gorell, J. (2006). Whole-body lifetime occupational lead exposure and risk of Parkinson's disease. *Environ Health Perspect* **114**, 1872-1876.

Factor-Litvak, P., Wasserman, G., Kline, J. K., and Graziano, J. (1999). The Yugoslavia Prospective Study of environmental lead exposure. *Environ Health Perspect* **107**, 9-15.

Hu, H., Rabinowitz, M., and Smith, D. (1998). Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. *Environ Health Perspect* **106**, 1-8.

Hu, H., Shih, R., Rothenberg, S., and Schwartz, B. S. (2007). The epidemiology of lead toxicity in adults: measuring dose and consideration of other methodologic issues. *Environ Health Perspect* **115**, 455-462.

Lanphear, B. P., Dietrich, K., Auinger, P., and Cox, C. (2000). Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. *Public Health Rep* **115**, 521-529.

Lanphear, B. P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D. C., Canfield, R. L., Dietrich, K. N., Bornschein, R., Greene, T., Rothenberg, S. J., Needleman, H. L., Schnaas, L., Wasserman, G., Graziano, J., and Roberts, R. (2005). Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect* **113**, 894-899.

Martin, D., Glass, T. A., Bandeen-Roche, K., Todd, A. C., Shi, W., and Schwartz, B. S. (2006). Association of blood lead and tibia lead with blood pressure and hypertension in a community sample of older adults. *Am J Epidemiol* **163**, 467-478.

Naicker, N., Norris, S. A., Mathee, A., Becker, P., and Richter, L. (2010). Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: findings from the Birth to Twenty cohort. *Sci Total Environ* **408**, 4949-4954.

Rabinowitz, M. B. (1991). Toxicokinetics of bone lead. *Environ Health Perspect* **91**, 33-37.

U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.

U.S. EPA (2011). Integrated Science Assessment for Lead (First External Review Draft). Office of Research and Development, National Center for Environmental Assessment-RTP Division, Research Triangle Park, NC.

9.2 Methods

ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
CDC (2005). Preventing lead Poisoning in Young Children, pp. 1-137. Centers for Disease Control and Prevention (CDC), Atlanta, GA.
CDC (2010). Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women: Recommendations from the Advisory Committee on Childhood Lead Poisoning Prevention. Centers for Disease Control and Prevention (CDC), Atlanta, GA.
U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.
U.S. EPA (2011). Integrated Science Assessment for Lead (First External Review Draft). Office of Research and Development, National Center for Environmental Assessment-RTP Division, Research Triangle Park, NC.

9.3 Exposure

ABLES (2009). Adult Blood Lead Epidemiology and Surveillance (ABLES) program case definition for an Elevated Blood Lead Level.
Alessio, L., Castoldi, M. R., Odone, P., and Franchini, I. (1981). Behaviour of indicators of exposure and effect after cessation of occupational exposure to lead. *Br J Ind Med* **38**, 262-267.
ATSDR (2001). Summary Report: Hair analysis panel discussion: Exploring the state of the science, pp. 1-199. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
Barbosa, F., Jr., Tanus-Santos, J. E., Gerlach, R. F., and Parsons, P. J. (2005). A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. *Environ Health Perspect* **113**, 1669-1674.
Bearer, C. F. (1995). How are children different from adults? *Environ Health Perspect* **103 Suppl 6**, 7-12.
Bellinger, D. C. (2008). Lead neurotoxicity and socioeconomic status: conceptual and analytical issues. *Neurotox* **29**, 828-832.
Benin, A. L., Sargent, J. D., Dalton, M., and Roda, S. (1999). High concentrations of heavy metals in neighborhoods near ore smelters in northern Mexico. *Environ Health Perspect* **107**, 279-284.
Berkowitz, Z., Price-Green, P., Bove, F. J., and Kaye, W. E. (2006). Lead exposure and birth outcomes in five communities in Shoshone County, Idaho. *Int J Hyg Environ Health* **209**, 123-132.
Bolger, P. M., Yess, N. J., Gunderson, E. L., Troxell, T. C., and Carrington, C. D. (1996). Identification and reduction of sources of dietary lead in the United States. *Food Addit Contam* **13**, 53-60.
Brody, D. J., Pirkle, J. L., Kramer, R. A., Flegal, K. M., Matte, T. D., Gunter, E. W., and Paschal, D. C. (1994). Blood lead levels in the US population. Phase 1 of the Third National Health and Nutrition Examination Survey (NHANES III, 1988 to 1991). *Jama* **272**, 277-283.
Brown, M. J., Hu, H., Gonzales-Cossio, T., Peterson, K. E., Sanin, L. H., de Luz Kageyama, M., Palazuelos, E., Aro, A., Schnaas, L., and Hernandez-Avila, M. (2000). Determinants of bone and blood lead concentrations in the early postpartum period. *Occup Environ Med* **57**, 535-541.
Buettner, C., Mukamal, K. J., Gardiner, P., Davis, R. B., Phillips, R. S., and Mittleman, M. A. (2009). Herbal supplement use and blood lead levels of United States adults. *J Gen Intern Med* **24**, 1175-1182.
Cavalleri, A., Minoia, C., Pozzoli, L., and Baruffini, A. (1978). Determination of plasma lead levels in normal subjects and in lead-exposed workers. *Br J Ind Med* **35**, 21-26.
CDC (1999). Adult Lead Poisoning from an Asian Remedy for Menstrual Cramps -- Connecticut, 1997. In Morbidity and Mortality Weekly Repor, Vol. 48 No. 02, pp. 27-29. Centers for Disease Control and Prevention (CDC), Atlanta, GA.
CDC (2002). Childhood Lead Poisoning Associated with Tamarind Candy and Folk Remedies - California, 1999-2000. In Morbidity and Mortality Weekly Repor, Vol. 51 No. 31, pp. 684-686. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2004). Blood lead levels in residents of homes with elevated lead in tap water--District of Columbia, 2004. In Morbidity and Mortality Weekly Report, Vol. 53 No. 12, pp. 268-270. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2005a). Blood lead levels--United States, 1999-2002. In Morbidity and Mortality Weekly Report, Vol. 54 No. 20, pp. 513-516. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2005b). Preventing lead Poisoning in Young Children, pp. 1-137. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2007). Interpreting and managing blood lead levels <10 ug/dl in children and reducing childhood exposures to lead: recommendations of CDC's Advisory Committee on Childhood Lead Poisoning Prevention. In Morbidity and Mortality Weekly Report, Vol. 56, pp. 1-15. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2009a). Children with elevated blood lead levels related to home renovation, repair, and painting activities --- New York State, 2006-2007. In Morbidity and Mortality Weekly Report, Vol. 58 No. 03, pp. 55-58. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2009b). Fourth National Report on Human Exposure to Environmental Chemicals. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2010). Guidelines for the Identification and Management of Lead Exposure in Pregnant and Lactating Women: Recommendations from the Advisory Committee on Childhood Lead Poisoning Prevention. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2011a). Adult blood lead epidemiology and surveillance --- United States, 2008--2009. In Morbidity and Mortality Weekly Report, Vol. 60 No. 35, pp. 841-845. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

CDC (2011b). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, February 2011. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Clark, S., Galke, W., Succop, P., Grote, J., McLaine, P., Wilson, J., Dixon, S., Menrath, W., Roda, S., Chen, M., Bornschein, R., and Jacobs, D. (2011). Effects of HUD-supported lead hazard control interventions in housing on children's blood lead. *Environ Res* **111**, 301-311.

Coon, S., Stark, A., Peterson, E., Gloi, A., Kortsha, G., Pounds, J., Chettle, D., and Gorell, J. (2006). Whole-body lifetime occupational lead exposure and risk of Parkinson's disease. *Environ Health Perspect* **114**, 1872-1876.

Edwards, M., Triantafyllidou, S., and Best, D. (2009). Elevated blood lead in young children due to lead-contaminated drinking water: Washington, DC, 2001-2004. *Environ Sci Technol* **43**, 1618-1623.

Factor-Litvak, P., Wasserman, G., Kline, J. K., and Graziano, J. (1999). The Yugoslavia Prospective Study of environmental lead exposure. *Environ Health Perspect* **107**, 9-15.

Fischbein, A., Wallace, J., Sassa, S., Kappas, A., Butts, G., Rohl, A., and Kaul, B. (1992). Lead poisoning from art restoration and pottery work: unusual exposure source and household risk. *J Environ Pathol Toxicol Oncol* **11**, 7-11.

Gelberg, K. H., and Fletcher, A. (2010). Adult blood lead reporting in New York State, 1994-2006. *Public Health Rep* **125**, 103-110.

Godoi, Q., Santos Jr, D., Nunes, L. C., Leme, F. O., Rufini, I. A., Agnelli, J. A. M., Trevizan, L. C., and Krug, F. J. (2009). Preliminary studies of laser-induced breakdown spectrometry for the determination of Ba, Cd, Cr and Pb in toys. *Spectrochimica Acta Part B: Atomic Spectroscopy* **64**, 573-581.

Graziano, J. H., and Blum, C. (1991). Lead exposure from lead crystal. *Lancet* **337**, 141-142.

Graziano, J. H., Popovac, D., Factor-Litvak, P., Shrout, P., Kline, J., Murphy, M. J., Zhao, Y. H., Mehmeti, A., Ahmedi, X., Rajovic, B., and et al. (1990). Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. *Environ Health Perspect* **89**, 95-100.

Greenway, J. A., and Gerstenberger, S. (2010). An Evaluation of Lead Contamination in Plastic Toys Collected from Day Care Centers in the Las Vegas Valley, Nevada, USA. *Bull Environ Contam Toxicol*.

Gulson, B. L., Mahaffey, K. R., Mizon, K. J., Korsch, M. J., Cameron, M. A., and Vimpani, G. (1995). Contribution of tissue lead to blood lead in adult female subjects based on stable lead isotope methods. *J Lab Clin Med* **125**, 703-712.

Gump, B. B., Reihman, J., Stewart, P., Lonky, E., Darvill, T., and Matthews, K. A. (2007). Blood lead (Pb) levels: a potential environmental mechanism explaining the relation between socioeconomic status and cardiovascular reactivity in children. *Health Psychol* **26**, 296-304.

Gump, B. B., Stewart, P., Reihman, J., Lonky, E., Darvill, T., Matthews, K. A., and Parsons, P. J. (2005). Prenatal and early childhood blood lead levels and cardiovascular functioning in 9(1/2) year old children. *Neurotoxicol Teratol* **27**, 655-665.

Haley, V. B., and Talbot, T. O. (2004). Geographic analysis of blood lead levels in New York State children born 1994-1997. *Environ Health Perspect* **112**, 1577-1582.

Handley, M. A., Hall, C., Sanford, E., Diaz, E., Gonzalez-Mendez, E., Drace, K., Wilson, R., Villalobos, M., and Croughan, M. (2007). Globalization, binational communities, and imported food risks: results of an outbreak investigation of lead poisoning in Monterey County, California. *Am J Public Health* **97**, 900-906.

Harkins, D. K., and Susten, A. S. (2003). Hair analysis: exploring the state of the science. *Environ Health Perspect* **111**, 576-578.

Hernandez-Avila, M., Smith, D., Meneses, F., Sanin, L. H., and Hu, H. (1998). The influence of bone and blood lead on plasma lead levels in environmentally exposed adults. *Environ Health Perspect* **106**, 473-477.

Hernandez Avila, M., Romieu, I., Rios, C., Rivero, A., and Palazuelos, E. (1991). Lead-glazed ceramics as major determinants of blood lead levels in Mexican women. *Environ Health Perspect* **94**, 117-120.

Hertz-Picciotto, I., Schramm, M., Watt-Morse, M., Chantala, K., Anderson, J., and Osterloh, J. (2000). Patterns and determinants of blood lead during pregnancy. *Am J Epidemiol* **152**, 829-837.

Hipkins, K. L., Materna, B. L., Payne, S. F., and Kirsch, L. C. (2004). Family lead poisoning associated with occupational exposure. *Clin Pediatr (Phila)* **43**, 845-849.

Hu, H., Rabinowitz, M., and Smith, D. (1998). Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. *Environ Health Perspect* **106**, 1-8.

Hu, H., Shih, R., Rothenberg, S., and Schwartz, B. S. (2007). The epidemiology of lead toxicity in adults: measuring dose and consideration of other methodologic issues. *Environ Health Perspect* **115**, 455-462.

James, H. M., Hilburn, M. E., and Blair, J. A. (1985). Effects of meals and meal times on uptake of lead from the gastrointestinal tract in humans. *Hum Toxicol* **4**, 401-407.

Jean Brown, M., Raymond, J., Homa, D., Kennedy, C., and Sinks, T. (2011). Association between children's blood lead levels, lead service lines, and water disinfection, Washington, DC, 1998-2006. *Environ Res* **111**, 67-74.

Jones, L., Parker, J. D., and Mendola, P. (2010). Blood lead and mercury levels in pregnant women in the United States, 2003-2008. *NCHS Data Brief*, 1-8.

Jones, R. L., Homa, D. M., Meyer, P. A., Brody, D. J., Caldwell, K. L., Pirkle, J. L., and Brown, M. J. (2009). Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988-2004. *Pediatrics* **123**, e376-385.

Kaufmann, R. B., Staes, C. J., and Matte, T. D. (2003). Deaths related to lead poisoning in the United States, 1979-1998. *Environ Res* **91**, 78-84.

Klitzman, S., Sharma, A., Nicaj, L., Vitkevich, R., and Leighton, J. (2002). Lead poisoning among pregnant women in New York City: risk factors and screening practices. *J Urban Health* **79**, 225-237.

Ko, R. J. (1998). Adulterants in Asian patent medicines. *N Engl J Med* **339**, 847.

Koyashiki, G. A., Paoliello, M. M., and Tchounwou, P. B. (2010). Lead levels in human milk and children's health risk: a systematic review. *Rev Environ Health* **25**, 243-253.

Kromhout, D., Wibowo, A. A., Herber, R. F., Dalderup, L. M., Heerdink, H., de Lezenne Coulander, C., and Zielhuis, R. L. (1985). Trace metals and coronary heart disease risk indicators in 152 elderly men (the Zutphen Study). *Am J Epidemiol* **122**, 378-385.

Labbe, R. F., Vreman, H. J., and Stevenson, D. K. (1999). Zinc protoporphyrin: A metabolite with a mission. *Clin Chem* **45**, 2060-2072.

Lanphear, B. P., Dietrich, K., Auinger, P., and Cox, C. (2000). Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. *Public Health Rep* **115**, 521-529.

Lanphear, B. P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D. C., Canfield, R. L., Dietrich, K. N., Bornschein, R., Greene, T., Rothenberg, S. J., Needleman, H. L., Schnaas, L., Wasserman, G., Graziano, J., and Roberts, R. (2005). Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect* **113**, 894-899.

Lanphear, B. P., Matte, T. D., Rogers, J., Clickner, R. P., Dietz, B., Bornschein, R. L., Succop, P., Mahaffey, K. R., Dixon, S., Galke, W., Rabinowitz, M., Farfel, M., Rohde, C., Schwartz, J., Ashley, P., and Jacobs, D. E. (1998). The contribution of lead-contaminated house dust and residential soil to children's blood lead levels. A pooled analysis of 12 epidemiologic studies. *Environ Res* **79**, 51-68.

Leggett, R. W. (1993). An age-specific kinetic model of lead metabolism in humans. *Environ Health Perspect* **101**, 598-616.

Lin, C. G., Schaider, L. A., Brabander, D. J., and Woolf, A. D. (2010). Pediatric lead exposure from imported Indian spices and cultural powders. *Pediatrics* **125**, e828-835.

Lockett, C. J., and Arbuckle, D. (1987). Lead, ferritin, zinc, and hypertension. *Bull Environ Contam Toxicol* **38**, 975-980.

Maddaloni, M., Lolacono, N., Manton, W., Blum, C., Drexler, J., and Graziano, J. (1998). Bioavailability of soilborne lead in adults, by stable isotope dilution. *Environ Health Perspect* **106 Suppl 6**, 1589-1594.

Mahaffey, K. R., Annest, J. L., Roberts, J., and Murphy, R. S. (1982). National estimates of blood lead levels: United States, 1976-1980: association with selected demographic and socioeconomic factors. *N Engl J Med* **307**, 573-579.

Manea-Krichten, M., Patterson, C., Miller, G., Settle, D., and Erel, Y. (1991). Comparative increases of lead and barium with age in human tooth enamel, rib and ulna. *Sci Total Environ* **107**, 179-203.

Mannino, D. M., Albalak, R., Grosse, S., and Repace, J. (2003). Second-hand smoke exposure and blood lead levels in U.S. children. *Epidemiology* **14**, 719-727.

Manton, W. I., Angle, C. R., Stanek, K. L., Kuntzelman, D., Reese, Y. R., and Kuehnemann, T. J. (2003). Release of lead from bone in pregnancy and lactation. *Environ Res* **92**, 139-151.

Martin, D., Glass, T. A., Bandeen-Roche, K., Todd, A. C., Shi, W., and Schwartz, B. S. (2006). Association of blood lead and tibia lead with blood pressure and hypertension in a community sample of older adults. *Am J Epidemiol* **163**, 467-478.

Matte, T. D., Proops, D., Palazuelos, E., Graef, J., and Hernandez Avila, M. (1994). Acute high-dose lead exposure from beverage contaminated by traditional Mexican pottery. *Lancet* **344**, 1064-1065.

Miranda, M. L., Kim, D., Hull, A. P., Paul, C. J., and Galeano, M. A. (2007). Changes in blood lead levels associated with use of chloramines in water treatment systems. *Environ Health Perspect* **115**, 221-225.

Morgan, B. W., Parramore, C. S., and Ethridge, M. (2004). Lead contaminated moonshine: a report of Bureau of Alcohol, Tobacco and Firearms analyzed samples. *Vet Hum Toxicol* **46**, 89-90.

Naicker, N., Norris, S. A., Mathee, A., Becker, P., and Richter, L. (2010). Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: findings from the Birth to Twenty cohort. *Sci Total Environ* **408**, 4949-4954.

Nie, L. H., Sanchez, S., Newton, K., Grodzins, L., Cleveland, R. O., and Weisskopf, M. G. (2011). In vivo quantification of lead in bone with a portable x-ray fluorescence system--methodology and feasibility. *Phys Med Biol* **56**, N39-51.

Park, S. K., Mukherjee, B., Xia, X., Sparrow, D., Weisskopf, M. G., Nie, H., and Hu, H. (2009). Bone lead level prediction models and their application to examine the relationship of lead exposure and hypertension in the Third National Health and Nutrition Examination Survey. *J Occup Environ Med* **51**, 1422-1436.

Parsons, P. J., Reilly, A. A., and Hussain, A. (1991). Observational study of erythrocyte protoporphyrin screening test for detecting low lead exposure in children: impact of lowering the blood lead action threshold. *Clin Chem* **37**, 216-225.

Pegues, D. A., Hughes, B. J., and Woernle, C. H. (1993). Elevated blood lead levels associated with illegally distilled alcohol. *Arch Intern Med* **153**, 1501-1504.

Rabinowitz, M. B. (1991). Toxicokinetics of bone lead. *Environ Health Perspect* **91**, 33-37.

Rickert, W. S., and Kaiserman, M. J. (1994). Levels of Lead, Cadmium, and Mercury in Canadian Cigarette Tobacco as Indicators of Environmental-Change - Results from a 21-Year Study (1968-1988). *Environmental Science & Technology* **28**, 924-927.

Rothenberg, S. J., Karchmer, S., Schnaas, L., Perroni, E., Zea, F., and Fernandez Alba, J. (1994). Changes in serial blood lead levels during pregnancy. *Environ Health Perspect* **102**, 876-880.

Rothenberg, S. J., Manalo, M., Jiang, J., Cuellar, R., Reyes, S., Sanchez, M., Diaz, M., Khan, F., Aguilar, A., Reynoso, B., Juaregui, M., Acosta, S., and Johnson, C. (1999a). Blood lead level and blood pressure during pregnancy in South Central Los Angeles. *Arch Environ Health* **54**, 382-389.

Rothenberg, S. J., Schnaas-Arrieta, L., Ugartechea, J. C., Perroni-Hernandez, E., Perez-Guerrero, I. A., Cansino-Prtiz, S., Salinas, V., Zea-Prado, F., and Chicz-Demet, A. (1992). A documented case of perinatal lead poisoning. *Am J Public Health* **82**, 613-614.

Rothenberg, S. J., Schnaas, L., Perroni, E., Hernandez, R. M., Martinez, S., and Hernandez, C. (1999b). Pre- and postnatal lead effect on head circumference: a case for critical periods. *Neurotoxicol Teratol* **21**, 1-11.

Sanborn, M. D., Abelsohn, A., Campbell, M., and Weir, E. (2002). Identifying and managing adverse environmental health effects: 3. Lead exposure. *CMAJ* **166**, 1287-1292.

Sanchez-Nazario, E. E., Mansilla-Rivera, I., Derieux-Cortes, J. C., Perez, C. M., and Rodriguez-Sierra, C. J. (2003). The association of lead-contaminated house dust and blood lead levels of children living on a former landfill in Puerto Rico. *P R Health Sci J* **22**, 153-159.

Schell, L. M., Czerwinski, S., Stark, A. D., Parsons, P. J., Gomez, M., and Samelson, R. (2000). Variation in blood lead and hematocrit levels during pregnancy in a socioeconomically disadvantaged population. *Arch Environ Health* **55**, 134-140.

Schell, L. M., Denham, M., Stark, A. D., Ravenscroft, J., Parsons, P., and Schulte, E. (2004). Relationship between blood lead concentration and dietary intakes of infants from 3 to 12 months of age. *Environ Res* **96**, 264-273.

Schnaas, L., Rothenberg, S. J., Flores, M. F., Martinez, S., Hernandez, C., Osorio, E., and Perroni, E. (2004). Blood lead secular trend in a cohort of children in Mexico City (1987-2002). *Environ Health Perspect* **112**, 1110-1115.

Seidel, S., Kreutzer, R., Smith, D., McNeel, S., and Gilliss, D. (2001). Assessment of commercial laboratories performing hair mineral analysis. *JAMA* **285**, 67-72.

Sexton, K. (1997). Sociodemographic aspects of human susceptibility to toxic chemicals: Do class and race matter for realistic risk assessment? *Environ Toxicol Pharmacol* **4**, 261-269.

Shannon, M. (2003). Severe lead poisoning in pregnancy. *Ambul Pediatr* **3**, 37-39.

Silbergeld, E. K., Schwartz, J., and Mahaffey, K. (1988). Lead and osteoporosis: mobilization of lead from bone in postmenopausal women. *Environ Res* **47**, 79-94.

Smith, D. R., Osterloh, J. D., and Flegal, A. R. (1996). Use of endogenous, stable lead isotopes to determine release of lead from the skeleton. *Environ Health Perspect* **104**, 60-66.

Snyder, J. E., Filipov, N. M., Parsons, P. J., and Lawrence, D. A. (2000). The efficiency of maternal transfer of lead and its influence on plasma IgE and splenic cellularity of mice. *Toxicol Sci* **57**, 87-94.

Strike, P. C., and Steptoe, A. (2004). Psychosocial factors in the development of coronary artery disease. *Progress in cardiovascular diseases* **46**, 337-347.

Svensson, B. G., Schutz, A., Nilsson, A., and Skerfving, S. (1992). Lead exposure in indoor firing ranges. *Int Arch Occup Environ Health* **64**, 219-221.

Telisman, S., Kersanc, A., and Prpic-Majic, D. (1982). The relevance of arguments for excluding ALAD from the recommended biological limit values in occupational exposure to inorganic lead (WHO 1980). *Int Arch Occup Environ Health* **50**, 397-412.

U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.

U.S. EPA (2007). Elevated Lead in D.C. Drinking Water - A study of Potential Causitive Events, Fional Summary Report, pp. 1-221. Office of Water, Washington, DC.

U.S. EPA (2011). Integrated Science Assessment for Lead (First External Review Draft). Office of Research and Development, National Center for Environmental Assessment-RTP Division, Research Triangle Park, NC.

Uryu, T., Yoshinaga, J., Yanagisawa, Y., Endo, M., and Takahashi, J. (2003). Analysis of lead in tooth enamel by laser ablation-inductively coupled plasma-mass spectrometry. *Anal Sci* **19**, 1413-1416.

VanArsdale, J. L., Leiker, R. D., Kohn, M., Merritt, T. A., and Horowitz, B. Z. (2004). Lead poisoning from a toy necklace. *Pediatrics* **114**, 1096-1099.

Wakefield, J. (2002). The lead effect? *Environ Health Perspect* **110**, A574-580.

Wibowo, A. A., Herber, R. F., Das, H. A., Roeleveld, N., and Zielhuis, R. L. (1986). Levels of metals in hair of young children as an indicator of environmental pollution. *Environ Res* **40**, 346-356.

Wildt, K., Berlin, M., and Isberg, P. E. (1987). Monitoring of zinc protoporphyrin levels in blood following occupational lead exposure. *Am J Ind Med* **12**, 385-398.

Yiin, L. M., Lu, S. E., Sannoh, S., Lim, B. S., and Rhoads, G. G. (2004). Evaluation of cleaning methods applied in home environments after renovation and remodeling activities. *Environ Res* **96**, 156-162.
Ziegler, E. E., Edwards, B. B., Jensen, R. L., Mahaffey, K. R., and Fomon, S. J. (1978). Absorption and retention of lead by infants. *Pediatr Res* **12**, 29-34.

9.4 Neurological Effects

Abbate, C., Buceti, R., Munao, F., Giorgianni, C., and Ferreri, G. (1995). Neurotoxicity induced by lead levels: an electrophysiological study. *Int Arch Occup Environ Health* **66**, 389-392.
Aguiar, A., Eubig, P. A., and Schantz, S. L. (2010). Attention deficit/hyperactivity disorder: a focused overview for children's environmental health researchers. *Environ Health Perspect* **118**, 1646-1653.
Al-Saleh, I., Nester, M., DeVol, E., Shinwari, N., Munchari, L., and Al-Shahria, S. (2001). Relationships between blood lead concentrations, intelligence, and academic achievement of Saudi Arabian schoolgirls. *Int J Hyg Environ Health* **204**, 165-174.
Al-Saleh, I., Nester, M., Mashhour, A., Moncari, L., Shinwari, N., Mohamed Gel, D., and Rabah, A. (2009). Prenatal and postnatal lead exposure and early cognitive development: longitudinal study in saudi arabia. *J Environ Pathol Toxicol Oncol* **28**, 283-302.
Altmann, L., Sveinsson, K., Kramer, U., Weishoff-Houben, M., Turfeld, M., Winneke, G., and Wiegand, H. (1998). Visual functions in 6-year-old children in relation to lead and mercury levels. *Neurotoxicol Teratol* **20**, 9-17.
ATSDR (2001). Summary Report: Hair analysis panel discussion: Exploring the state of the science, pp. 1-199. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
Baghurst, P. A., McMichael, A. J., Wigg, N. R., Vimpani, G. V., Robertson, E. F., Roberts, R. J., and Tong, S. L. (1992). Environmental exposure to lead and children's intelligence at the age of seven years. The Port Pirie Cohort Study. *N Engl J Med* **327**, 1279-1284.
Bandeen-Roche, K., Glass, T. A., Bolla, K. I., Todd, A. C., and Schwartz, B. S. (2009). Cumulative lead dose and cognitive function in older adults. *Epidemiology* **20**, 831-839.
Barbeito, A. G., Martinez-Palma, L., Vargas, M. R., Pehar, M., Manay, N., Beckman, J. S., Barbeito, L., and Cassina, P. (2010). Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS. *Neurobiol Dis* **37**, 574-580.
Basha, M. R., Wei, W., Bakheet, S. A., Benitez, N., Siddiqi, H. K., Ge, Y. W., Lahiri, D. K., and Zawia, N. H. (2005). The fetal basis of amyloidogenesis: exposure to lead and latent overexpression of amyloid precursor protein and beta-amyloid in the aging brain. *J Neurosci* **25**, 823-829.
Bellinger, D., Hu, H., Titlebaum, L., and Needleman, H. L. (1994a). Attentional correlates of dentin and bone lead levels in adolescents. *Arch Environ Health* **49**, 98-105.
Bellinger, D., Leviton, A., Allred, E., and Rabinowitz, M. (1994b). Pre- and postnatal lead exposure and behavior problems in school-aged children. *Environ Res* **66**, 12-30.
Bellinger, D., Leviton, A., Needleman, H. L., Waternaux, C., and Rabinowitz, M. (1986). Low-level lead exposure and infant development in the first year. *Neurobehav Toxicol Teratol* **8**, 151-161.
Bellinger, D., Leviton, A., and Sloman, J. (1990). Antecedents and correlates of improved cognitive performance in children exposed in utero to low levels of lead. *Environ Health Perspect* **89**, 5-11.
Bellinger, D., Leviton, A., Waternaux, C., Needleman, H., and Rabinowitz, M. (1987). Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. *N Engl J Med* **316**, 1037-1043.
Bellinger, D., Sloman, J., Leviton, A., Rabinowitz, M., Needleman, H. L., and Waternaux, C. (1991). Low-level lead exposure and children's cognitive function in the preschool years. *Pediatrics* **87**, 219-227.
Bellinger, D. C., and Needleman, H. L. (2003). Intellectual impairment and blood lead levels. *N Engl J Med* **349**, 500-502; author reply 500-502.
Bellinger, D. C., Needleman, H. L., Leviton, A., Waternaux, C., Rabinowitz, M. B., and Nichols, M. L. (1984). Early sensory-motor development and prenatal exposure to lead. *Neurobehav Toxicol Teratol* **6**, 387-402.

Bellinger, D. C., Stiles, K. M., and Needleman, H. L. (1992). Low-level lead exposure, intelligence and academic achievement: a long-term follow-up study. *Pediatrics* **90**, 855-861.

Bleecker, M. L., Ford, D. P., Lindgren, K. N., Scheetz, K., and Tiburzi, M. J. (2003). Association of chronic and current measures of lead exposure with different components of brainstem auditory evoked potentials. *Neurotox* **24**, 625-631.

Booze, R. M., Mactutus, C. F., Annau, Z., and Tilson, H. A. (1983). Neonatal triethyl lead neurotoxicity in rat pups: initial behavioral observations and quantification. *Neurobehav Toxicol Teratol* **5**, 367-375.

Bouchard, M. F., Bellinger, D. C., Weuve, J., Matthews-Bellinger, J., Gilman, S. E., Wright, R. O., Schwartz, J., and Weisskopf, M. G. (2009). Blood lead levels and major depressive disorder, panic disorder, and generalized anxiety disorder in US young adults. *Arch Gen Psychiatry* **66**, 1313-1319.

Boucher, O., Muckle, G., Saint-Amour, D., Dewailly, E., Ayotte, P., Jacobson, S. W., Jacobson, J. L., and Bastien, C. H. (2009). The relation of lead neurotoxicity to the event-related potential P3b component in Inuit children from arctic Quebec. *Neurotox* **30**, 1070-1077.

Braun, J. M., Froehlich, T. E., Daniels, J. L., Dietrich, K. N., Hornung, R., Auinger, P., and Lanphear, B. P. (2008). Association of environmental toxicants and conduct disorder in U.S. children: NHANES 2001-2004. *Environ Health Perspect* **116**, 956-962.

Braun, J. M., Kahn, R. S., Froehlich, T., Auinger, P., and Lanphear, B. P. (2006). Exposures to environmental toxicants and attention deficit hyperactivity disorder in U.S. children. *Environ Health Perspect* **114**, 1904-1909.

Burns, J. M., Baghurst, P. A., Sawyer, M. G., McMichael, A. J., and Tong, S. L. (1999). Lifetime low-level exposure to environmental lead and children's emotional and behavioral development at ages 11-13 years. The Port Pirie Cohort Study. *Am J Epidemiol* **149**, 740-749.

Canfield, R. L., Henderson, C. R., Jr., Cory-Slechta, D. A., Cox, C., Jusko, T. A., and Lanphear, B. P. (2003a). Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *N Engl J Med* **348**, 1517-1526.

Canfield, R. L., Kreher, D. A., Cornwell, C., and Henderson, C. R., Jr. (2003b). Low-level lead exposure, executive functioning, and learning in early childhood. *Child Neuropsychol* **9**, 35-53.

CDC (2005). Preventing lead Poisoning in Young Children, pp. 1-137. Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Chandramouli, K., Steer, C. D., Ellis, M., and Emond, A. M. (2009). Effects of early childhood lead exposure on academic performance and behaviour of school age children. *Arch Dis Child* **94**, 844-848.

Chen, A., Cai, B., Dietrich, K. N., Radcliffe, J., and Rogan, W. J. (2007). Lead exposure, IQ, and behavior in urban 5- to 7-year-olds: does lead affect behavior only by lowering IQ? *Pediatrics* **119**, e650-e658.

Chiodo, L. M., Covington, C., Sokol, R. J., Hannigan, J. H., Jannise, J., Ager, J., Greenwald, M., and Delaney-Black, V. (2007). Blood lead levels and specific attention effects in young children. *Neurotoxicol Teratol* **29**, 538-546.

Chiodo, L. M., Jacobson, S. W., and Jacobson, J. L. (2004). Neurodevelopmental effects of postnatal lead exposure at very low levels. *Neurotoxicol Teratol* **26**, 359-371.

Cho, S. C., Kim, B. N., Hong, Y. C., Shin, M. S., Yoo, H. J., Kim, J. W., Bhang, S. Y., Cho, I. H., and Kim, H. W. (2010). Effect of environmental exposure to lead and tobacco smoke on inattentive and hyperactive symptoms and neurocognitive performance in children. *J Child Psychol Psychiatry* **51**, 1050-1057.

Chuang, H. Y., Kuo, C. H., Chiu, Y. W., Ho, C. K., Chen, C. J., and Wu, T. N. (2007). A case-control study on the relationship of hearing function and blood concentrations of lead, manganese, arsenic, and selenium. *Sci Total Environ* **387**, 79-85.

Coon, S., Stark, A., Peterson, E., Gloi, A., Kortsha, G., Pounds, J., Chettle, D., and Gorell, J. (2006). Whole-body lifetime occupational lead exposure and risk of Parkinson's disease. *Environ Health Perspect* **114**, 1872-1876.

Cooney, G. H., Bell, A., McBride, W., and Carter, C. (1989a). Low-level exposures to lead: the Sydney lead study. *Dev Med Child Neurol* **31**, 640-649.

Cooney, G. H., Bell, A., McBride, W., and Carter, C. (1989b). Neurobehavioural consequences of prenatal low level exposures to lead. *Neurotoxicol Teratol* **11**, 95-104.

Dietrich, K. N., Berger, O. G., and Succop, P. A. (1993a). Lead exposure and the motor developmental status of urban six-year-old children in the Cincinnati Prospective Study. *Pediatrics* **91**, 301-307.

Dietrich, K. N., Berger, O. G., Succop, P. A., Hammond, P. B., and Bornschein, R. L. (1993b). The developmental consequences of low to moderate prenatal and postnatal lead exposure: intellectual attainment in the Cincinnati Lead Study Cohort following school entry. *Neurotoxicol Teratol* **15**, 37-44.

Dietrich, K. N., Krafft, K. M., Bornschein, R. L., Hammond, P. B., Berger, O., Succop, P. A., and Bier, M. (1987). Low-level fetal lead exposure effect on neurobehavioral development in early infancy. *Pediatrics* **80**, 721-730.

Dietrich, K. N., Ris, M. D., Succop, P. A., Berger, O. G., and Bornschein, R. L. (2001). Early exposure to lead and juvenile delinquency. *Neurotoxicol Teratol* **23**, 511-518.

Dietrich, K. N., Succop, P. A., Berger, O. G., and Keith, R. W. (1992). Lead exposure and the central auditory processing abilities and cognitive development of urban children: the Cincinnati Lead Study cohort at age 5 years. *Neurotoxicol Teratol* **14**, 51-56.

Dietrich, K. N., Succop, P. A., Bornschein, R. L., Krafft, K. M., Berger, O., Hammond, P. B., and Buncher, C. R. (1990). Lead exposure and neurobehavioral development in later infancy. *Environ Health Perspect* **89**, 13-19.

Dogu, O., Louis, E. D., Tamer, L., Unal, O., Yilmaz, A., and Kaleagasi, H. (2007). Elevated blood lead concentrations in essential tremor: a case-control study in Mersin, Turkey. *Environ Health Perspect* **115**, 1564-1568.

Dyatlov, V. A., and Lawrence, D. A. (2002). Neonatal lead exposure potentiates sickness behavior induced by Listeria monocytogenes infection of mice. *Brain Behav Immun* **16**, 477-492.

Ernhart, C. B., Morrow-Tlucak, M., Marler, M. R., and Wolf, A. W. (1987). Low level lead exposure in the prenatal and early preschool periods: early preschool development. *Neurotoxicol Teratol* **9**, 259-270.

Ernhart, C. B., Morrow-Tlucak, M., and Wolf, A. W. (1988). Low level lead exposure and intelligence in the preschool years. *Sci Total Environ* **71**, 453-459.

Eubig, P. A., Aguiar, A., and Schantz, S. L. (2010). Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environ Health Perspect* **118**, 1654-1667.

Factor-Litvak, P., Wasserman, G., Kline, J. K., and Graziano, J. (1999). The Yugoslavia Prospective Study of environmental lead exposure. *Environ Health Perspect* **107**, 9-15.

Fang, F., Kwee, L. C., Allen, K. D., Umbach, D. M., Ye, W., Watson, M., Keller, J., Oddone, E. Z., Sandler, D. P., Schmidt, S., and Kamel, F. (2010). Association between blood lead and the risk of amyotrophic lateral sclerosis. *Am J Epidemiol* **171**, 1126-1133.

Fergusson, D. M., Boden, J. M., and Horwood, L. J. (2008). Dentine lead levels in childhood and criminal behaviour in late adolescence and early adulthood. *J Epidemiol Community Health* **62**, 1045-1050.

Fergusson, D. M., Fergusson, J. E., Horwood, L. J., and Kinzett, N. G. (1988). A longitudinal study of dentine lead levels, intelligence, school performance and behaviour. Part II. Dentine lead and cognitive ability. *J Child Psychol Psychiatry* **29**, 793-809.

Fergusson, D. M., Horwood, L. J., and Lynskey, M. T. (1993). Early dentine lead levels and subsequent cognitive and behavioural development. *J Child Psychol Psychiatry* **34**, 215-227.

Fergusson, D. M., Horwood, L. J., and Lynskey, M. T. (1997). Early dentine lead levels and educational outcomes at 18 years. *J Child Psychol Psychiatry* **38**, 471-478.

Forst, L. S., Freels, S., and Persky, V. (1997). Occupational lead exposure and hearing loss. *J Occup Environ Med* **39**, 658-660.

Fox, D. A., and Boyles, W. K. (2007). Toxic resposnes of the ocular and visual system. In Casarett and Doull's Toxicology: The Science of Poisons K. CD, ed., pp. 655-697. McGraw-Hill, New York, NY.

Fox, D. A., Kala, S. V., Hamilton, W. R., Johnson, J. E., and O'Callaghan, J. P. (2008). Low-level human equivalent gestational lead exposure produces supernormal scotopic electroretinograms, increased retinal neurogenesis, and decreased retinal dopamine utilization in rats. *Environ Health Perspect* **116**, 618-625.

Froehlich, T. E., Lanphear, B. P., Auinger, P., Hornung, R., Epstein, J. N., Braun, J., and Kahn, R. S. (2009). Association of tobacco and lead exposures with attention-deficit/hyperactivity disorder. *Pediatrics* **124**, e1054-e1063.

Gao, S., Jin, Y., Unverzagt, F. W., Ma, F., Hall, K. S., Murrell, J. R., Cheng, Y., Shen, J., Ying, B., Ji, R., Matesan, J., Liang, C., and Hendrie, H. C. (2008). Trace element levels and cognitive function in rural elderly Chinese. *J Gerontol A Biol Sci Med Sci* **63**, 635-641.

Glass, T. A., Bandeen-Roche, K., McAtee, M., Bolla, K., Todd, A. C., and Schwartz, B. S. (2009). Neighborhood psychosocial hazards and the association of cumulative lead dose with cognitive function in older adults. *Am J Epidemiol* **169**, 683-692.

Gomaa, A., Hu, H., Bellinger, D., Schwartz, J., Tsaih, S. W., Gonzalez-Cossio, T., Schnaas, L., Peterson, K., Aro, A., and Hernandez-Avila, M. (2002). Maternal bone lead as an independent risk factor for fetal neurotoxicity: a prospective study. *Pediatrics* **110**, 110-118.

Ha, M., Kwon, H. J., Lim, M. H., Jee, Y. K., Hong, Y. C., Leem, J. H., Sakong, J., Bae, J. M., Hong, S. J., Roh, Y. M., and Jo, S. J. (2009). Low blood levels of lead and mercury and symptoms of attention deficit hyperactivity in children: a report of the children's health and environment research (CHEER). *Neurotox* **30**, 31-36.

Hornung, R. W., Lanphear, B. P., and Dietrich, K. N. (2009). Age of greatest susceptibility to childhood lead exposure: a new statistical approach. *Environ Health Perspect* **117**, 1309-1312.

Hu, H., Tellez-Rojo, M. M., Bellinger, D., Smith, D., Ettinger, A. S., Lamadrid-Figueroa, H., Schwartz, J., Schnaas, L., Mercado-Garcia, A., and Hernandez-Avila, M. (2006). Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. *Environ Health Perspect* **114**, 1730-1735.

Hubbs-Tait, L., Kennedy, T. S., Droke, E. A., Belanger, D. M., and Parker, J. R. (2007). Zinc, iron, and lead: relations to head start children's cognitive scores and teachers' ratings of behavior. *J Am Diet Assoc* **107**, 128-133.

Hwang, Y. H., Chiang, H. Y., Yen-Jean, M. C., and Wang, J. D. (2009). The association between low levels of lead in blood and occupational noise-induced hearing loss in steel workers. *Sci Total Environ* **408**, 43-49.

Iwata, T., Yano, E., Karita, K., Dakeishi, M., and Murata, K. (2005). Critical dose of lead affecting postural balance in workers. *Am J Ind Med* **48**, 319-325.

Jedrychowski, W., Perera, F., Jankowski, J., Mrozek-Budzyn, D., Mroz, E., Flak, E., Edwards, S., Skarupa, A., and Lisowska-Miszczyk, I. (2009a). Gender specific differences in neurodevelopmental effects of prenatal exposure to very low-lead levels: the prospective cohort study in three-year olds. *Early Hum Dev* **85**, 503-510.

Jedrychowski, W., Perera, F. P., Jankowski, J., Mrozek-Budzyn, D., Mroz, E., Flak, E., Edwards, S., Skarupa, A., and Lisowska-Miszczyk, I. (2009b). Very low prenatal exposure to lead and mental development of children in infancy and early childhood: Krakow prospective cohort study. *Neuroepidemiology* **32**, 270-278.

Jusko, T. A., Henderson, C. R., Lanphear, B. P., Cory-Slechta, D. A., Parsons, P. J., and Canfield, R. L. (2008). Blood lead concentrations < 10 microg/dL and child intelligence at 6 years of age. *Environ Health Perspect* **116**, 243-248.

Kamel, F., Umbach, D. M., Hu, H., Munsat, T. L., Shefner, J. M., Taylor, J. A., and Sandler, D. P. (2005). Lead exposure as a risk factor for amyotrophic lateral sclerosis. *Neuro-degenerative diseases* **2**, 195-201.

Kamel, F., Umbach, D. M., Lehman, T. A., Park, L. P., Munsat, T. L., Shefner, J. M., Sandler, D. P., Hu, H., and Taylor, J. A. (2003). Amyotrophic lateral sclerosis, lead, and genetic susceptibility: polymorphisms in the delta-aminolevulinic acid dehydratase and vitamin D receptor genes. *Environ Health Perspect* **111**, 1335-1339.

Kamel, F., Umbach, D. M., Munsat, T. L., Shefner, J. M., Hu, H., and Sandler, D. P. (2002). Lead exposure and amyotrophic lateral sclerosis. *Epidemiology* **13**, 311-319.

Kamel, F., Umbach, D. M., Stallone, L., Richards, M., Hu, H., and Sandler, D. P. (2008). Association of lead exposure with survival in amyotrophic lateral sclerosis. *Environ Health Perspect* **116**, 943-947.

Kim, Y., Cho, S. C., Kim, B. N., Hong, Y. C., Shin, M. S., Yoo, H. J., Kim, J. W., and Bhang, S. Y. (2010). Association between blood lead levels (<5 μg/dL) and inattention-hyperactivity and neurocognitive profiles in school-aged Korean children. *Sci Total Environ* **408**, 5737-5743.

Kim, Y., Kim, B. N., Hong, Y. C., Shin, M. S., Yoo, H. J., Kim, J. W., Bhang, S. Y., and Cho, S. C. (2009). Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. *Neurotox* **30**, 564-571.

Kordas, K., Canfield, R. L., Lopez, P., Rosado, J. L., Vargas, G. G., Cebrian, M. E., Rico, J. A., Ronquillo, D., and Stoltzfus, R. J. (2006). Deficits in cognitive function and achievement in Mexican first-graders with low blood lead concentrations. *Environ Res* **100**, 371-386.

Krieg, E. F., Jr., and Butler, M. A. (2009). Blood lead, serum homocysteine, and neurobehavioral test performance in the third National Health and Nutrition Examination Survey. *Neurotox* **30**, 281-289.

Krieg, E. F., Jr., Butler, M. A., Chang, M. H., Liu, T., Yesupriya, A., Dowling, N., and Lindegren, M. L. (2010). Lead and cognitive function in VDR genotypes in the third National Health and Nutrition Examination Survey. *Neurotoxicol Teratol* **32**, 262-272.

Krieg, E. F., Jr., Butler, M. A., Chang, M. H., Liu, T., Yesupriya, A., Lindegren, M. L., and Dowling, N. (2009). Lead and cognitive function in ALAD genotypes in the third National Health and Nutrition Examination Survey. *Neurotoxicol Teratol* **31**, 364-371.

Krieg, E. F., Jr., Chrislip, D. W., Crespo, C. J., Brightwell, W. S., Ehrenberg, R. L., and Otto, D. A. (2005). The relationship between blood lead levels and neurobehavioral test performance in NHANES III and related occupational studies. *Public Health Rep* **120**, 240-251.

Lanphear, B. P., Dietrich, K., Auinger, P., and Cox, C. (2000). Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. *Public Health Rep* **115**, 521-529.

Lanphear, B. P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D. C., Canfield, R. L., Dietrich, K. N., Bornschein, R., Greene, T., Rothenberg, S. J., Needleman, H. L., Schnaas, L., Wasserman, G., Graziano, J., and Roberts, R. (2005). Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect* **113**, 894-899.

Leviton, A., Bellinger, D., Allred, E. N., Rabinowitz, M., Needleman, H., and Schoenbaum, S. (1993). Pre- and postnatal low-level lead exposure and children's dysfunction in school. *Environ Res* **60**, 30-43.

Louis, E. D., Applegate, L., Graziano, J. H., Parides, M., Slavkovich, V., and Bhat, H. K. (2005). Interaction between blood lead concentration and delta-amino-levulinic acid dehydratase gene polymorphisms increases the odds of essential tremor. *Mov Disord* **20**, 1170-1177.

Louis, E. D., Factor-Litvak, P., Gerbin, M., Slavkovich, V., Graziano, J. H., Jiang, W., and Zheng, W. (2011). Blood harmane, blood lead, and severity of hand tremor: Evidence of additive effects. *Neurotox* **32**, 227-232.

Louis, E. D., Jurewicz, E. C., Applegate, L., Factor-Litvak, P., Parides, M., Andrews, L., Slavkovich, V., Graziano, J. H., Carroll, S., and Todd, A. (2003). Association between essential tremor and blood lead concentration. *Environ Health Perspect* **111**, 1707-1711.

Marcus, D. K., Fulton, J. J., and Clarke, E. J. (2010). Lead and conduct problems: a meta-analysis. *J Clin Child Adolesc Psychol* **39**, 234-241.

Marlowe, M., and Bliss, L. B. (1993). Hair element concentrations and young children's classroom and home behavior. *Journal of Orthomolecular Medicine* **8**, 79-88.

Marlowe, M., and Errera, J. (1982). Low lead levels and behavior problems in children. *Behavioral Disorders* **7**, 163-172.

Marlowe, M., Stellern, J., Moon, C., and Errera, J. (1985). Main and interaction effects of metallic toxins on aggressive classroom behavior. *Agressive Behavior* **11**, 41-48.

Min, M. O., Singer, L. T., Kirchner, H. L., Minnes, S., Short, E., Hussain, Z., and Nelson, S. (2009). Cognitive development and low-level lead exposure in poly-drug exposed children. *Neurotoxicol Teratol* **31**, 225-231.

Miranda, M. L., Kim, D., Galeano, M. A., Paul, C. J., Hull, A. P., and Morgan, S. P. (2007). The relationship between early childhood blood lead levels and performance on end-of-grade tests. *Environ Health Perspect* **115**, 1242-1247.

Miranda, M. L., Kim, D., Reiter, J., Overstreet Galeano, M. A., and Maxson, P. (2009). Environmental contributors to the achievement gap. *Neurotox* **30**, 1019-1024.

Muldoon, S. B., Cauley, J. A., Kuller, L. H., Morrow, L., Needleman, H. L., Scott, J., and Hooper, F. J. (1996). Effects of blood lead levels on cognitive function of older women. *Neuroepidemiology* **15**, 62-72.

Needleman, H. L., and Gatsonis, C. A. (1990). Low-level lead exposure and the IQ of children. A meta-analysis of modern studies. *Jama* **263**, 673-678.

Needleman, H. L., McFarland, C., Ness, R. B., Fienberg, S. E., and Tobin, M. J. (2002). Bone lead levels in adjudicated delinquents. A case control study. *Neurotoxicol Teratol* **24**, 711-717.

Needleman, H. L., Riess, J. A., Tobin, M. J., Biesecker, G. E., and Greenhouse, J. B. (1996). Bone lead levels and delinquent behavior. *Jama* **275**, 363-369.

Needleman, H. L., Schell, A., Bellinger, D., Leviton, A., and Allred, E. N. (1990). The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. *N Engl J Med* **322**, 83-88.

Nicolescu, R., Petcu, C., Cordeanu, A., Fabritius, K., Schlumpf, M., Krebs, R., Kramer, U., and Winneke, G. (2010). Environmental exposure to lead, but not other neurotoxic metals, relates to core elements of ADHD in Romanian children: performance and questionnaire data. *Environ Res* **110**, 476-483.

Nigg, J. T. (2008). ADHD, lead exposure and prevention: how much lead or how much evidence is needed? *Expert Rev Neurother* **8**, 519-521.

Nigg, J. T., Knottnerus, G. M., Martel, M. M., Nikolas, M., Cavanagh, K., Karmaus, W., and Rappley, M. D. (2008). Low blood lead levels associated with clinically diagnosed attention-deficit/hyperactivity disorder and mediated by weak cognitive control. *Biol Psychiatry* **63**, 325-331.

Nigg, J. T., Nikolas, M., Mark Knottnerus, G., Cavanagh, K., and Friderici, K. (2010). Confirmation and extension of association of blood lead with attention-deficit/hyperactivity disorder (ADHD) and ADHD symptom domains at population-typical exposure levels. *J Child Psychol Psychiatry* **51**, 58-65.

Nordberg, M., Winblad, B., Fratiglioni, L., and Basun, H. (2000). Lead concentrations in elderly urban people related to blood pressure and mental performance: results from a population-based study. *Am J Ind Med* **38**, 290-294.

Osman, K., Pawlas, K., Schutz, A., Gazdzik, M., Sokal, J. A., and Vahter, M. (1999). Lead exposure and hearing effects in children in Katowice, Poland. *Environ Res* **80**, 1-8.

Otto, D., Robinson, G., Baumann, S., Schroeder, S., Mushak, P., Kleinbaum, D., and Boone, L. (1985). 5-year follow-up study of children with low-to-moderate lead absorption: electrophysiological evaluation. *Environ Res* **38**, 168-186.

Otto, D. A., and Fox, D. A. (1993). Auditory and visual dysfunction following lead exposure. *Neurotox* **14**, 191-207.

Park, S. K., Elmarsafawy, S., Mukherjee, B., Spiro, A., 3rd, Vokonas, P. S., Nie, H., Weisskopf, M. G., Schwartz, J., and Hu, H. (2010). Cumulative lead exposure and age-related hearing loss: the VA Normative Aging Study. *Hear Res* **269**, 48-55.

Payton, M., Riggs, K. M., Spiro, A., 3rd, Weiss, S. T., and Hu, H. (1998). Relations of bone and blood lead to cognitive function: the VA Normative Aging Study. *Neurotoxicol Teratol* **20**, 19-27.

Peters, J. L., Weisskopf, M. G., Spiro, A., Schwartz, J., Sparrow, D., Nie, H., Hu, H., Wright, R. O., and Wright, R. J. (2010). Interaction of Stress, Lead Burden, and Age on Cognition in Older Men: The VA Normative Aging Study. *Environ Health Perspect* **118**, 505-510.

Pilsner, J. R., Hu, H., Wright, R. O., Kordas, K., Ettinger, A. S., Sanchez, B. N., Cantonwine, D., Lazarus, A. L., Cantoral, A., Schnaas, L., Tellez-Rojo, M. M., and Hernandez-Avila, M. (2010). Maternal MTHFR genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City. *Am J Clin Nutr* **92**, 226-234.

Pocock, S. J., Smith, M., and Baghurst, P. (1994). Environmental lead and children's intelligence: a systematic review of the epidemiological evidence. *Bmj* **309**, 1189-1197.

Rabinowitz, M. B., Wang, J. D., and Soong, W. T. (1992). Children's classroom behavior and lead in Taiwan. *Bull Environ Contam Toxicol* **48**, 282-288.

Rajan, P., Kelsey, K. T., Schwartz, J. D., Bellinger, D. C., Weuve, J., Sparrow, D., Spiro, A., 3rd, Smith, T. J., Nie, H., Hu, H., and Wright, R. O. (2007). Lead burden and psychiatric symptoms and the modifying influence of the delta-aminolevulinic acid dehydratase (ALAD) polymorphism: the VA Normative Aging Study. *Am J Epidemiol* **166**, 1400-1408.

Rajan, P., Kelsey, K. T., Schwartz, J. D., Bellinger, D. C., Weuve, J., Spiro, A., 3rd, Sparrow, D., Smith, T. J., Nie, H., Weisskopf, M. G., Hu, H., and Wright, R. O. (2008). Interaction of the delta-aminolevulinic acid dehydratase polymorphism and lead burden on cognitive function: the VA normative aging study. *J Occup Environ Med* **50**, 1053-1061.

Rhodes, D., Spiro, A., 3rd, Aro, A., and Hu, H. (2003). Relationship of bone and blood lead levels to psychiatric symptoms: the normative aging study. *J Occup Environ Med* **45**, 1144-1151.

Ris, M. D., Dietrich, K. N., Succop, P. A., Berger, O. G., and Bornschein, R. L. (2004). Early exposure to lead and neuropsychological outcome in adolescence. *J Int Neuropsychol Soc* **10**, 261-270.

Robinson, G., Baumann, S., Kleinbaum, D., Barton, C., Schroeder, S. R., Mushak, P., and Otto, D. A. (1985). Effects of low to moderate lead exposure on brainstem auditory evoked potentials in children. In Neurobehavioral Methods in Occupational and Environmental Health: Environ. Health Doc 3, pp. 177-182. WHO, Copenhagen.

Rothenberg, S. J., Poblano, A., and Garza-Morales, S. (1994). Prenatal and perinatal low level lead exposure alters brainstem auditory evoked responses in infants. *Neurotox* **15**, 695-699.

Rothenberg, S. J., Poblano, A., and Schnaas, L. (2000). Brainstem auditory evoked response at five years and prenatal and postnatal blood lead. *Neurotoxicol Teratol* **22**, 503-510.

Rothenberg, S. J., and Rothenberg, J. C. (2005). Testing the dose-response specification in epidemiology: public health and policy consequences for lead. *Environ Health Perspect* **113**, 1190-1195.

Rothenberg, S. J., Schnaas, L., Salgado-Valladares, M., Casanueva, E., Geller, A. M., Hudnell, H. K., and Fox, D. A. (2002). Increased ERG a- and b-wave amplitudes in 7- to 10-year-old children resulting from prenatal lead exposure. *Invest Ophthalmol Vis Sci* **43**, 2036-2044.

Roy, A., Bellinger, D., Hu, H., Schwartz, J., Ettinger, A. S., Wright, R. O., Bouchard, M., Palaniappan, K., and Balakrishnan, K. (2009). Lead exposure and behavior among young children in Chennai, India. *Environ Health Perspect* **117**, 1607-1611.

Schnaas, L., Rothenberg, S. J., Flores, M. F., Martinez, S., Hernandez, C., Osorio, E., Velasco, S. R., and Perroni, E. (2006). Reduced intellectual development in children with prenatal lead exposure. *Environ Health Perspect* **114**, 791-797.

Schnaas, L., Rothenberg, S. J., Perroni, E., Martinez, S., Hernandez, C., and Hernandez, R. M. (2000). Temporal pattern in the effect of postnatal blood lead level on intellectual development of young children. *Neurotoxicol Teratol* **22**, 805-810.

Schwartz, J. (1994). Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold. *Environ Res* **65**, 42-55.

Schwartz, J., and Otto, D. (1987). Blood lead, hearing thresholds, and neurobehavioral development in children and youth. *Arch Environ Health* **42**, 153-160.

Schwartz, J., and Otto, D. (1991). Lead and minor hearing impairment. *Arch Environ Health* **46**, 300-305.

Shen, X. M., Yan, C. H., Guo, D., Wu, S. M., Li, R. Q., Huang, H., Ao, L. M., Zhou, J. D., Hong, Z. Y., Xu, J. D., Jin, X. M., and Tang, J. M. (1998). Low-level prenatal lead exposure and neurobehavioral development of children in the first year of life: a prospective study in Shanghai. *Environ Res* **79**, 1-8.

Shih, R. A., Glass, T. A., Bandeen-Roche, K., Carlson, M. C., Bolla, K. I., Todd, A. C., and Schwartz, B. S. (2006). Environmental lead exposure and cognitive function in community-dwelling older adults. *Neurology* **67**, 1556-1562.

Silva, P. A., Hughes, P., Williams, S., and Faed, J. M. (1988). Blood lead, intelligence, reading attainment, and behaviour in eleven year old children in Dunedin, New Zealand. *J Child Psychol Psychiatry* **29**, 43-52.

Solon, O., Riddell, T. J., Quimbo, S. A., Butrick, E., Aylward, G. P., Lou Bacate, M., and Peabody, J. W. (2008). Associations between cognitive function, blood lead concentration, and nutrition among children in the central Philippines. *J Pediatr* **152**, 237-243.

Surkan, P. J., Schnaas, L., Wright, R. J., Tellez-Rojo, M. M., Lamadrid-Figueroa, H., Hu, H., Hernandez-Avila, M., Bellinger, D. C., Schwartz, J., Perroni, E., and Wright, R. O. (2008). Maternal self-esteem, exposure to lead, and child neurodevelopment. *Neurotox* **29**, 278-285.

Surkan, P. J., Zhang, A., Trachtenberg, F., Daniel, D. B., McKinlay, S., and Bellinger, D. C. (2007). Neuropsychological function in children with blood lead levels <10 microg/dL. *Neurotox* **28**, 1170-1107.

Tellez-Rojo, M. M., Bellinger, D. C., Arroyo-Quiroz, C., Lamadrid-Figueroa, H., Mercado-Garcia, A., Schnaas-Arrieta, L., Wright, R. O., Hernandez-Avila, M., and Hu, H. (2006). Longitudinal associations between blood lead concentrations lower than 10 microg/dL and neurobehavioral development in environmentally exposed children in Mexico City. *Pediatrics* **118**, e323-e330.

Thomson, G. O., Raab, G. M., Hepburn, W. S., Hunter, R., Fulton, M., and Laxen, D. P. (1989). Blood-lead levels and children's behaviour--results from the Edinburgh Lead Study. *J Child Psychol Psychiatry* **30**, 515-528.

Tong, S., Baghurst, P., McMichael, A., Sawyer, M., and Mudge, J. (1996). Lifetime exposure to environmental lead and children's intelligence at 11-13 years: the Port Pirie cohort study. *Bmj* **312**, 1569-1575.

U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.

U.S. EPA (2007). Elevated Lead in D.C. Drinking Water - A study of Potential Causitive Events, Fional Summary Report, pp. 1-221. Office of Water, Washington, DC.

U.S. EPA (2011). Integrated Science Assessment for Lead (First External Review Draft). Office of Research and Development, National Center for Environmental Assessment-RTP Division, Research Triangle Park, NC.

van Wijngaarden, E., Campbell, J. R., and Cory-Slechta, D. A. (2009). Bone lead levels are associated with measures of memory impairment in older adults. *Neurotox* **30**, 572-580.

Vinceti, M., Guidetti, D., Bergomi, M., Caselgrandi, E., Vivoli, R., Olmi, M., Rinaldi, L., Rovesti, S., and Solime, F. (1997). Lead, cadmium, and selenium in the blood of patients with sporadic amyotrophic lateral sclerosis. *Ital J Neurol Sci* **18**, 87-92.

Walkowiak, J., Altmann, L., Kramer, U., Sveinsson, K., Turfeld, M., Weishoff-Houben, M., and Winneke, G. (1998). Cognitive and sensorimotor functions in 6-year-old children in relation to lead and mercury levels: adjustment for intelligence and contrast sensitivity in computerized testing. *Neurotoxicol Teratol* **20**, 511-521.

Wang, C. L., Chuang, H. Y., Ho, C. K., Yang, C. Y., Tsai, J. L., Wu, T. S., and Wu, T. N. (2002). Relationship between blood lead concentrations and learning achievement among primary school children in Taiwan. *Environ Res* **89**, 12-18.

Wang, F. T., Hu, H., Schwartz, J., Weuve, J., Spiro, A. S., Sparrow, D., Nie, H., Silverman, E. K., Weiss, S. T., and Wright, R. O. (2007). Modifying effects of the HFE polymorphisms on the association between lead burden and cognitive decline. *Environ Health Perspect* **115**, 1210-1215.

Wang, H. L., Chen, X. T., Yang, B., Ma, F. L., Wang, S., Tang, M. L., Hao, M. G., and Ruan, D. Y. (2008). Case-control study of blood lead levels and attention deficit hyperactivity disorder in Chinese children. *Environ Health Perspect* **116**, 1401-1406.

Wasserman, G. A., Factor-Litvak, P., Liu, X., Todd, A. C., Kline, J. K., Slavkovich, V., Popovac, D., and Graziano, J. H. (2003). The relationship between blood lead, bone lead and child intelligence. *Child Neuropsychol* **9**, 22-34.

Wasserman, G. A., Liu, X., Lolacono, N. J., Factor-Litvak, P., Kline, J. K., Popovac, D., Morina, N., Musabegovic, A., Vrenezi, N., Capuni-Paracka, S., Lekic, V., Preteni-Redjepi, E., Hadzialjevic, S., Slavkovich, V., and Graziano, J. H. (1997). Lead exposure and intelligence in 7-year-old children: the Yugoslavia Prospective Study. *Environ Health Perspect* **105**, 956-962.

Wasserman, G. A., Liu, X., Pine, D. S., and Graziano, J. H. (2001). Contribution of maternal smoking during pregnancy and lead exposure to early child behavior problems. *Neurotoxicol Teratol* **23**, 13-21.

Wasserman, G. A., Musabegovic, A., Liu, X., Kline, J., Factor-Litvak, P., and Graziano, J. H. (2000). Lead exposure and motor functioning in 4(1/2)-year-old children: the Yugoslavia prospective study. *J Pediatr* **137**, 555-561.

Wasserman, G. A., Staghezza-Jaramillo, B., Shrout, P., Popovac, D., and Graziano, J. (1998). The effect of lead exposure on behavior problems in preschool children. *Am J Public Health* **88**, 481-486.

Weisskopf, M. G., Proctor, S. P., Wright, R. O., Schwartz, J., Spiro, A., 3rd, Sparrow, D., Nie, H., and Hu, H. (2007). Cumulative lead exposure and cognitive performance among elderly men. *Epidemiology* **18**, 59-66.

Weisskopf, M. G., Weuve, J., Nie, H., Saint-Hilaire, M. H., Sudarsky, L., Simon, D. K., Hersh, B., Schwartz, J., Wright, R. O., and Hu, H. (2010). Association of Cumulative Lead Exposure with Parkinson's Disease. *Environ Health Perspect* **118**, 1609-1613.

Weisskopf, M. G., Wright, R. O., Schwartz, J., Spiro, A., 3rd, Sparrow, D., Aro, A., and Hu, H. (2004). Cumulative lead exposure and prospective change in cognition among elderly men: the VA Normative Aging Study. *Am J Epidemiol* **160**, 1184-1193.

Weuve, J., Kelsey, K. T., Schwartz, J., Bellinger, D., Wright, R. O., Rajan, P., Spiro, A., 3rd, Sparrow, D., Aro, A., and Hu, H. (2006). Delta-aminolevulinic acid dehydratase polymorphism and the relation between low level lead exposure and the Mini-Mental Status Examination in older men: the Normative Aging Study. *Occup Environ Med* **63**, 746-753.

Weuve, J., Korrick, S. A., Weisskopf, M. G., Ryan, L. M., Schwartz, J., Nie, H., Grodstein, F., and Hu, H. (2009). Cumulative exposure to lead in relation to cognitive function in older women. *Environ Health Perspect* **117**, 574-580.

Wright, J. P., Dietrich, K. N., Ris, M. D., Hornung, R. W., Wessel, S. D., Lanphear, B. P., Ho, M., and Rae, M. N. (2008). Association of prenatal and childhood blood lead concentrations with criminal arrests in early adulthood. *PLoS Med* **5**, e101.

Wright, R. O., Tsaih, S. W., Schwartz, J., Spiro, A., 3rd, McDonald, K., Weiss, S. T., and Hu, H. (2003). Lead exposure biomarkers and mini-mental status exam scores in older men. *Epidemiology* **14**, 713-718.

Yule, W., Urbanowicz, M.-A., Lansdown, R., and Millar, I. B. (1984). Teachers' ratings of children's behaviour in relation to blood lead levels. *British Journal of Developmental Psychology* **2**, 295-305.

Zawia, N. H., and Basha, M. R. (2005). Environmental risk factors and the developmental basis for Alzheimer's disease. *Rev Neurosci* **16**, 325-337.

9.5 Immune Effects

Ahmad Al Obaidi, A. H., Mohamed Al Samarai, A. G., Yahya Al Samarai, A. K., and Al Janabi, J. M. (2008). The predictive value of IgE as biomarker in asthma. *J Asthma* **45**, 654-663.

Annesi-Maesano, I., Pollitt, R., King, G., Bousquet, J., Hellier, G., Sahuquillo, J., and Huel, G. (2003). In utero exposure to lead and cord blood total IgE. Is there a connection? *Allergy* **58**, 589-594.

ATSDR (2001). Summary Report: Hair analysis panel discussion: Exploring the state of the science, pp. 1-199. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Basaran, N., and Undeger, U. (2000). Effects of lead on immune parameters in occupationally exposed workers. *Am J Ind Med* **38**, 349-354.

Beeh, K. M., Ksoll, M., and Buhl, R. (2000). Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. *Eur Respir J* **16**, 609-614.

Belles-Isles, M., Ayotte, P., Dewailly, E., Weber, J. P., and Roy, R. (2002). Cord blood lymphocyte functions in newborns from a remote maritime population exposed to organochlorines and methylmercury. *J Toxicol Environ Health A* **65**, 165-182.

Bener, A., Almehdi, A. M., Alwash, R., and Al-Neamy, F. R. (2001). A pilot survey of blood lead levels in various types of workers in the United Arab Emirates. *Environ Int* **27**, 311-314.

Blakley, B. R., and Archer, D. L. (1981). The effect of lead acetate on the immune response in mice. *Toxicol Appl Pharmacol* **61**, 18-26.

Boscolo, P., Di Gioacchino, M., Sabbioni, E., Benvenuti, F., Conti, P., Reale, M., Bavazzano, P., and Giuliano, G. (1999). Expression of lymphocyte subpopulations, cytokine serum levels, and blood and urinary trace elements in asymptomatic atopic men exposed to an urban environment. *Int Arch Occup Environ Health* **72**, 26-32.

Boscolo, P., Di Gioacchino, M., Sabbioni, E., Di Giacomo, F., Reale, M., Volpe, A. R., Di Sciascio, M. B., Conti, P., and Giuliano, G. (2000). Lymphocyte subpopulations, cytokines and trace elements in asymptomatic atopic women exposed to an urban environment. *Life Sci* **67**, 1119-1126.

Chen, S., Golemboski, K., Piepenbrink, M., and Dietert, R. (2004). Developmental immunotoxicity of lead in the rat: influence of maternal diet. *J Toxicol Environ Health A* **67**, 495-511.

Choi, J. W., and Kim, S. K. (2005). Relationships of lead, copper, zinc, and cadmium levels versus hematopoiesis and iron parameters in healthy adolescents. *Ann Clin Lab Sci* **35**, 428-434.

Corsini, E., and Kimber, I. (2007). Factors governing susceptibility to chemical allergy. *Toxicol Lett* **168**, 255-259.

De Swert, L. F. (1999). Risk factors for allergy. *Eur J Pediatr* **158**, 89-94.

Dietert, R. R. (2008). Developmental immunotoxicology (DIT): windows of vulnerability, immune dysfunction and safety assessment. *J Immunotoxicol* **5**, 401-412.

Dietert, R. R., and Piepenbrink, M. S. (2006). Lead and immune function. *Crit Rev Toxicol* **36**, 359-385.

Dyatlov, V. A., and Lawrence, D. A. (2002). Neonatal lead exposure potentiates sickness behavior induced by Listeria monocytogenes infection of mice. *Brain Behav Immun* **16**, 477-492.

Faith, R. E., Luster, M. I., and Kimmel, C. A. (1979). Effect of chronic developmental lead exposure on cell-mediated immune functions. *Clin Exp Immunol* **35**, 413-420.

Fischbein, A., Tsang, P., Luo, J. C., Roboz, J. P., Jiang, J. D., and Bekesi, J. G. (1993). Phenotypic aberrations of CD3+ and CD4+ cells and functional impairments of lymphocytes at low-level occupational exposure to lead. *Clin Immunol Immunopathol* **66**, 163-168.

Gao, D., Mondal, T. K., and Lawrence, D. A. (2007). Lead effects on development and function of bone marrow-derived dendritic cells promote Th2 immune responses. *Toxicol Appl Pharmacol* **222**, 69-79.

Garcia-Leston, J., Roma-Torres, J., Vilares, M., Pinto, R., Cunha, L. M., Prista, J., Teixeira, J. P., Mayan, O., Pasaro, E., Mendez, J., and Laffon, B. (2011). Biomonitoring of a population of Portuguese workers exposed to lead. *Mutat Res* **721**, 81-88.

Governa, M., Valentino, M., and Visona, I. (1987). In vitro impairment of human granulocyte functions by lead. *Arch Toxicol* **59**, 421-425.

Governa, M., Valentino, M., Visona, I., and Scielso, R. (1988). Impairment of chemotaxis of polymorphonuclear leukocytes from lead acid battery workers. *Sci Total Environ* **71**, 543-546.

Guillard, O., and Lauwerys, R. (1989). In vitro and in vivo effect of mercury, lead and cadmium on the generation of chemiluminescence by human whole blood. *Biochem Pharmacol* **38**, 2819-2823.

Guo, T. L., Mudzinski, S. P., and Lawrence, D. A. (1996). The heavy metal lead modulates the expression of both TNF-alpha and TNF-alpha receptors in lipopolysaccharide-activated human peripheral blood mononuclear cells. *J Leukoc Biol* **59**, 932-939.

Hegazy, R. M., Hamdy, R., and Kamel, H. F. (2011). Modulation of IgE levels in lead exposed children by parental cigarette smoking, qualyobia governate, Egypt. *Int J Pharm Bio Sci* **2**, 272-385.

Heinrich, J., Hoelscher, B., Wjst, M., Ritz, B., Cyrys, J., and Wichmann, H. (1999). Respiratory diseases and allergies in two polluted areas in East Germany. *Environ Health Perspect* **107**, 53-62.

Hemdan, N. Y., Emmrich, F., Adham, K., Wichmann, G., Lehmann, I., El-Massry, A., Ghoneim, H., Lehmann, J., and Sack, U. (2005). Dose-dependent modulation of the in vitro cytokine production of human immune competent cells by lead salts. *Toxicol Sci* **86**, 75-83.

Heo, Y., Lee, B. K., Ahn, K. D., and Lawrence, D. A. (2004). Serum IgE elevation correlates with blood lead levels in battery manufacturing workers. *Hum Exp Toxicol* **23**, 209-213.

Hon, K. L., Ching, G. K., Hung, E. C., and Leung, T. F. (2009). Serum lead levels in childhood eczema. *Clin Exp Dermatol* **34**, e508-e509.

Hon, K. L., Wang, S. S., Hung, E. C., Lam, H. S., Lui, H. H., Chow, C. M., Ching, G. K., Fok, T. F., Ng, P. C., and Leung, T. F. (2010). Serum levels of heavy metals in childhood eczema and skin diseases: friends or foes. *Pediatr Allergy Immunol* **21**, 831-836.

Hon, K. L. E. (2011). Concerning heavy metals in childhood eczema. *Pediatr Allergy Immunol* **22**, 343-343.

Horiguchi, S., Kiyota, I., Endo, G., Teramoto, K., Shinagawa, K., Wakitani, F., Konishi, Y., Kiyota, A., Ota, A., Tanaka, H., and et al. (1992). Serum immunoglobulin and complement C3 levels in workers exposed to lead. *Osaka City Med J* **38**, 149-153.

Hunninghake, G. M., Chu, J. H., Sharma, S. S., Cho, M. H., Himes, B. E., Rogers, A. J., Murphy, A., Carey, V. J., and Raby, B. A. (2011). The CD4+ T-cell transcriptome and serum IgE in asthma: IL17RB and the role of sex. *BMC Pulmonary Medicine* **11**, 17.

Hunninghake, G. M., Lasky-Su, J., Soto-Quiros, M. E., Avila, L., Liang, C., Lake, S. L., Hudson, T. J., Spesny, M., Fournier, E., Sylvia, J. S., Freimer, N. B., Klanderman, B. J., Raby, B. A., and Celedon, J. C. (2008). Sex-stratified linkage analysis identifies a female-specific locus for IgE to cockroach in Costa Ricans. *Am J Respir Crit Care Med* **177**, 830-836.

Izaks, G. J., Remarque, E. J., Becker, S. V., and Westendorp, R. G. (2003). Lymphocyte count and mortality risk in older persons. The Leiden 85-Plus Study. *J Am Geriatr Soc* **51**, 1461-1465.

Jarvis, D., Luczynska, C., Chinn, S., Potts, J., Sunyer, J., Janson, C., Svanes, C., Künzli, N., Leynaert, B., Heinrich, J., Kerkhof, M., Ackermann-Liebrich, U., Antó, J. M., Cerveri, I., de Marco, R., Gislason, T., Neukirch, F., Vermeire, P., Wjst, M., and Burney, P. (2005). Change in prevalence of IgE sensitization and mean total IgE with age and cohort. *The Journal of allergy and clinical immunology* **116**, 675-682.

Jedrychowski, W., Perera, F., Maugeri, U., Miller, R. L., Rembiasz, M., Flak, E., Mroz, E., Majewska, R., and Zembala, M. (2011). Intrauterine exposure to lead may enhance sensitization to common inhalant allergens in early childhood. A prospective prebirth cohort study. *Environ Res* **111**, 119-124.

Joseph, C. L., Havstad, S., Ownby, D. R., Peterson, E. L., Maliarik, M., McCabe, M. J., Jr., Barone, C., and Johnson, C. C. (2005). Blood lead level and risk of asthma. *Environ Health Perspect* **113**, 900-904.

Karmaus, W., Brooks, K. R., Nebe, T., Witten, J., Obi-Osius, N., and Kruse, H. (2005). Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study. *Environ Health* **4**, 5.

Kelly, C., and Gangur, V. (2009). Sex Disparity in Food Allergy: Evidence from the PubMed Database. *J Allergy (Cairo)* **2009**, 159845.

Kotaniemi-Syrjanen, A., Reijonen, T. M., Korhonen, K., and Korppi, M. (2002). Wheezing requiring hospitalization in early childhood: predictive factors for asthma in a six-year follow-up. *Pediatr Allergy Immunol* **13**, 418-425.

Kuo, H. W., Hsiao, T. Y., and Lai, J. S. (2001). Immunological effects of long-term lead exposure among Taiwanese workers. *Arch Toxicol* **75**, 569-573.

Leggett, R. W. (1993). An age-specific kinetic model of lead metabolism in humans. *Environ Health Perspect* **101**, 598-616.

Li, S., Zhengyan, Z., Rong, L., and Hanyun, C. (2005). Decrease of CD4+ T-lymphocytes in children exposed to environmental lead. *Biol Trace Elem Res* **105**, 19-25.

Luebke, R., Chen, D., Dietert, R., Yang, Y., and Luster, M. (2006). Immune system maturity and sensitivity to chemical exposure. *J Toxicol Environ Health A* **69**, 811-825.

Luster, M. I., Faith, R. E., and Kimmel, C. A. (1978). Depression of humoral immunity in rats following chronic developmental lead exposure. *J Environ Pathol Toxicol* **1**, 397-402.

Luster, M. I., Portier, C., Pait, D. G., White, K. L., Jr., Gennings, C., Munson, A. E., and Rosenthal, G. J. (1992). Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam Appl Toxicol* **18**, 200-210.

Lutz, P., Gale, N., Hewett, J., Phillips, P., Looney, F., and Bengsch, H. (1992). The effect of lead on the immune system of children. *Trace Substances in Environmental Health XXV, Suppl. Environ. Geochem. Health* **14**, 129-144.

Lutz, P. M., Wilson, T. J., Ireland, J., Jones, A. L., Gorman, J. S., Gale, N. L., Johnson, J. C., and Hewett, J. E. (1999). Elevated immunoglobulin E (IgE) levels in children with exposure to environmental lead. *Toxicology* **134**, 63-78.

McCabe, M. J., Jr., Singh, K. P., and Reiners, J. J., Jr. (1999). Lead intoxication impairs the generation of a delayed type hypersensitivity response. *Toxicology* **139**, 255-264.

Mediaty, A., and Neuber, K. (2005). Total and specific serum IgE decreases with age in patients with allergic rhinitis, asthma and insect allergy but not in patients with atopic dermatitis. *Immun Ageing* **2**, 9.

Min, J. Y., Min, K. B., Kim, R., Cho, S. I., and Paek, D. (2008). Blood lead levels and increased bronchial responsiveness. *Biol Trace Elem Res* **123**, 41-46.

Mishra, K. P., Rani, R., Yadav, V. S., and Naik, S. (2010). Effect of lead exposure on lymphocyte subsets and activation markers. *Immunopharmacol Immunotoxicol* **32**, 446-449.

Mudzinski, S. P., Rudofsky, U. H., Mitchell, D. G., and Lawrence, D. A. (1986). Analysis of lead effects on in vivo antibody-mediated immunity in several mouse strains. *Toxicol Appl Pharmacol* **83**, 321-330.

Myers, S. N., Rowell, B., and Binns, H. J. (2002). Lead poisoning and asthma: an examination of comorbidity. *Arch Pediatr Adolesc Med* **156**, 863-866.

Narita, S., Goldblum, R. M., Watson, C. S., Brooks, E. G., Estes, D. M., Curran, E. M., and Midoro-Horiuti, T. (2007). Environmental estrogens induce mast cell degranulation and enhance IgE-mediated release of allergic mediators. *Environ Health Perspect* **115**, 48-52.

Parronchi, P., Brugnolo, F., Sampognaro, S., and Maggi, E. (2000). Genetic and environmental factors contributing to the onset of allergic disorders. *Int Arch Allergy Immunol* **121**, 2-9.

Pineda-Zavaleta, A. P., Garcia-Vargas, G., Borja-Aburto, V. H., Acosta-Saavedra, L. C., Vera Aguilar, E., Gomez-Munoz, A., Cebrian, M. E., and Calderon-Aranda, E. S. (2004). Nitric oxide and superoxide anion production in monocytes from children exposed to arsenic and lead in region Lagunera, Mexico. *Toxicol Appl Pharmacol* **198**, 283-290.

Pinkerton, L. E., Biagini, R. E., Ward, E. M., Hull, R. D., Deddens, J. A., Boeniger, M. F., Schnorr, T. M., MacKenzie, B. A., and Luster, M. I. (1998). Immunologic findings among lead-exposed workers. *Am J Ind Med* **33**, 400-408.

Pizent, A., Macan, J., Jurasovic, J., Varnai, V. M., Milkovic-Kraus, S., and Kanceljak-Macan, B. (2008). Association of toxic and essential metals with atopy markers and ventilatory lung function in women and men. *Sci Total Environ* **390**, 369-376.

Pugh Smith, P., and Nriagu, J. O. (2011). Lead poisoning and asthma among low-income and African American children in Saginaw, Michigan. *Environ Res* **111**, 81-86.

Queiroz, M. L., Almeida, M., Gallao, M. I., and Hoehr, N. F. (1993). Defective neutrophil function in workers occupationally exposed to lead. *Pharmacol Toxicol* **72**, 73-77.

Queiroz, M. L., Costa, F. F., Bincoletto, C., Perlingeiro, R. C., Dantas, D. C., Cardoso, M. P., and Almeida, M. (1994). Engulfment and killing capabilities of neutrophils and phagocytic splenic function in persons occupationally exposed to lead. *Int J Immunopharmacol* **16**, 239-244.

Rabinowitz, M. B., Allred, E. N., Bellinger, D. C., Leviton, A., and Needleman, H. L. (1990). Lead and childhood propensity to infectious and allergic disorders: is there an association? *Bull Environ Contam Toxicol* **44**, 657-660.

Raby, B. A., Soto-Quiros, M. E., Avila, L., Lake, S. L., Murphy, A., Liang, C., Fournier, E., Spesny, M., Sylvia, J. S., Verner, A., Hudson, T. J., Klanderman, B. J., Freimer, N. B., Silverman, E. K., and Celedon, J. C. (2007). Sex-specific linkage to total serum immunoglobulin E in families of children with asthma in Costa Rica. *Hum Mol Genet* **16**, 243-253.

Reigart, J. R., and Graber, C. D. (1976). Evaluation of the humoral immune response of children with low lead exposure. *Bull Environ Contam Toxicol* **16**, 112-117.

Sarasua, S. M., Vogt, R. F., Henderson, L. O., Jones, P. A., and Lybarger, J. A. (2000). Serum immunoglobulins and lymphocyte subset distributions in children and adults living in communities assessed for lead and cadmium exposure. *J Toxicol Environ Health A* **60**, 1-15.

Sata, F., Araki, S., Tanigawa, T., Morita, Y., Sakurai, S., Nakata, A., and Katsuno, N. (1998). Changes in T cell subpopulations in lead workers. *Environ Res* **76**, 61-64.

Snyder, J. E., Filipov, N. M., Parsons, P. J., and Lawrence, D. A. (2000). The efficiency of maternal transfer of lead and its influence on plasma IgE and splenic cellularity of mice. *Toxicol Sci* **57**, 87-94.

Sun, L., Hu, J., Zhao, Z., Li, L., and Cheng, H. (2003). Influence of exposure to environmental lead on serum immunoglobulin in preschool children. *Environ Res* **92**, 124-128.

Tryphonas, H. (2001). Approaches to detecting immunotoxic effects of environmental contaminants in humans. *Environ Health Perspect* **109 Suppl 6**, 877-884.

U.S. EPA (1998). Health Effects Test Guidelines: OPPTS 870.7800 Immunotoxicity. In Report, pp. 1-11. Office of Prevention, Pesticides and Toxic Substances, Washington, DC.

U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.

U.S. EPA (2011). Integrated Science Assessment for Lead (First External Review Draft). Office of Research and Development, National Center for Environmental Assessment-RTP Division, Research Triangle Park, NC.

Undeger, U., Basaran, N., Canpinar, H., and Kansu, E. (1996). Immune alterations in lead-exposed workers. *Toxicology* **109**, 167-172.

Villanueva, M. B., Koizumi, S., and Jonai, H. (2000). Cytokine production by human peripheral blood mononuclear cells after exposure to heavy metals. *Journal of Health Science* **46**, 358-362.

Vukmanovic-Stejic, M., Reed, J. R., Lacy, K. E., Rustin, M. H., and Akbar, A. N. (2006). Mantoux Test as a model for a secondary immune response in humans. *Immunol Lett* **107**, 93-101.

Wagnerova, M., Wagner, V., Madlo, Z., Zavazal, V., Wokounova, D., Kriz, J., and Mohyla, O. (1986). Seasonal variations in the level of immunoglobulins and serum proteins of children differing by exposure to air-borne lead. *J Hyg Epidemiol Microbiol Immunol* **30**, 127-138.

Welshons, W. V., Thayer, K. A., Judy, B. M., Taylor, J. A., Curran, E. M., and vom Saal, F. S. (2003). Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* **111**, 994-1006.

Zhao, Z. Y., Li, R., Sun, L., Li, Z. Y., and Yang, R. L. (2004). Effect of lead exposure on the immune function of lymphocytes and erythrocytes in preschool children. *J Zhejiang Univ Sci* **5**, 1001-1004.

9.6 Cardiovascular Effects

Afridi, H. I., Kazi, T. G., Kazi, N., Kandhro, G. A., Baig, J. A., Shah, A. Q., Jamali, M. K., and Arain, M. B. (2010). Evaluation of toxic elements in scalp hair samples of myocardial infarction patients at different stages as related to controls. *Biol Trace Elem Res* **134**, 1-12.

Al-Saleh, I., Shinwari, N., Mashhour, A., Mohamed Gel, D., Ghosh, M. A., Shammasi, Z., and Al-Nasser, A. (2005). Is lead considered as a risk factor for high blood pressure during menopause period among Saudi women? *Int J Hyg Environ Health* **208**, 341-356.

Apostoli, P., Maranelli, G., Dei Cas, L., and Micciolo, R. (1990). Blood lead and blood pressure: a cross sectional study in a general population group. *Cardiologia* **35**, 597-603.

ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Bakhtiarian, A., Dizaji, R., Mohaghegh, A., Immami-Khansari, F., and Ghazi-Khansari, M. (2006). The study of blood lead concentration in hypertensive and normotensive adults in Tehran's hospitals. *Journal of Medical Sciences* **6**, 103-107.

Chen, A., Rhoads, G. G., Cai, B., Salganik, M., and Rogan, W. J. (2006). The effect of chelation on blood pressure in lead-exposed children: a randomized study. *Environ Health Perspect* **114**, 579-583.

Cheng, Y., Schwartz, J., Sparrow, D., Aro, A., Weiss, S. T., and Hu, H. (2001). Bone lead and blood lead levels in relation to baseline blood pressure and the prospective development of hypertension: the Normative Aging Study. *Am J Epidemiol* **153**, 164-171.

Cheng, Y., Schwartz, J., Vokonas, P. S., Weiss, S. T., Aro, A., and Hu, H. (1998). Electrocardiographic conduction disturbances in association with low-level lead exposure (the Normative Aging Study). *Am J Cardiol* **82**, 594-599.

Chu, N. F., Liou, S. H., Wu, T. N., and Chang, P. Y. (1999). Reappraisal of the relation between blood lead concentration and blood pressure among the general population in Taiwan. *Occup Environ Med* **56**, 30-33.

Den Hond, E., Nawrot, T., and Staessen, J. A. (2002). The relationship between blood pressure and blood lead in NHANES III. National Health and Nutritional Examination Survey. *J Hum Hypertens* **16**, 563-568.

Dolenc, P., Staessen, J. A., Lauwerys, R. R., and Amery, A. (1993). Short report: low-level lead exposure does not increase the blood pressure in the general population. Cadmibel Study Group. *J Hypertens* **11**, 589-593.

Elmarsafawy, S. F., Jain, N. B., Schwartz, J., Sparrow, D., Nie, H., and Hu, H. (2006). Dietary calcium as a potential modifier of the relationship of lead burden to blood pressure. *Epidemiology* **17**, 531-537.

Eum, K.-D., Nie, L. H., Schwartz, J., Vokonas, P. S., Sparrow, D., Hu, H., and Weisskopf, M. G. (2011). Prospective Cohort Study of Lead Exposure and Electrocardiographic Conduction Disturbances in the Department of Veterans Affairs Normative Aging Study. *Environ Health Perspect* **in press**.

Factor-Litvak, P., Kline, J. K., Popovac, D., Hadzialjevic, S., Lekic, V., Preteni-Rexhepi, E., Capuni-Paracka, S., Slavkovich, V., and Graziano, J. (1996). Blood lead and blood pressure in young children. *Epidemiology* **7**, 633-637.

Factor-Litvak, P., Wasserman, G., Kline, J. K., and Graziano, J. (1999). The Yugoslavia Prospective Study of environmental lead exposure. *Environ Health Perspect* **107**, 9-15.

Gartside, P. S. (1988). The relationship of blood lead levels and blood pressure in NHANES II: additional calculations. *Environ Health Perspect* **78**, 31-34.

Gerr, F., Letz, R., Stokes, L., Chettle, D., McNeill, F., and Kaye, W. (2002). Association between bone lead concentration and blood pressure among young adults. *Am J Ind Med* **42**, 98-106.

Glenn, B. S., Stewart, W. F., Links, J. M., Todd, A. C., and Schwartz, B. S. (2003). The longitudinal association of lead with blood pressure. *Epidemiology* **14**, 30-36.

Grandjean, P., Hollnagel, H., Hedegaard, L., Christensen, J. M., and Larsen, S. (1989). Blood lead-blood pressure relations: alcohol intake and hemoglobin as confounders. *Am J Epidemiol* **129**, 732-739.

Guallar, E., Silbergeld, E. K., Navas-Acien, A., Malhotra, S., Astor, B. C., Sharrett, A. R., and Schwartz, B. S. (2006). Confounding of the relation between homocysteine and peripheral arterial disease by lead, cadmium, and renal function. *Am J Epidemiol* **163**, 700-708.

Gulson, B. L., Jameson, C. W., Mahaffey, K. R., Mizon, K. J., Korsch, M. J., and Vimpani, G. (1997). Pregnancy increases mobilization of lead from maternal skeleton. *J Lab Clin Med* **130**, 51-62.

Gump, B. B., Mackenzie, J. A., Bendinskas, K., Morgan, R., Dumas, A. K., Palmer, C. D., and Parsons, P. J. (2011). Low-level Pb and cardiovascular responses to acute stress in children: the role of cardiac autonomic regulation. *Neurotoxicol Teratol* **33**, 212-219.

Gump, B. B., Reihman, J., Stewart, P., Lonky, E., Darvill, T., and Matthews, K. A. (2007). Blood lead (Pb) levels: a potential environmental mechanism explaining the relation between socioeconomic status and cardiovascular reactivity in children. *Health Psychol* **26**, 296-304.

Gump, B. B., Stewart, P., Reihman, J., Lonky, E., Darvill, T., Matthews, K. A., and Parsons, P. J. (2005). Prenatal and early childhood blood lead levels and cardiovascular functioning in 9(1/2) year old children. *Neurotoxicol Teratol* **27**, 655-665.

Hense, H. W., Filipiak, B., and Keil, U. (1993). The association of blood lead and blood pressure in population surveys. *Epidemiology* **4**, 173-179.

Hense, H. W., Filipiak, B., and Keil, U. (1994). Alcohol consumption as a modifier of the relation between blood lead and blood pressure. *Epidemiology* **5**, 120-123.

Hu, H., Aro, A., Payton, M., Korrick, S., Sparrow, D., Weiss, S. T., and Rotnitzky, A. (1996). The relationship of bone and blood lead to hypertension. The Normative Aging Study. *Jama* **275**, 1171-1176.

Ishida, M., Ishizaki, M., and Yamada, Y. (1996). Decreases in postural change in finger blood flow in ceramic painters chronically exposed to low level lead. *Am J Ind Med* **29**, 547-553.

Jain, N. B., Potula, V., Schwartz, J., Vokonas, P. S., Sparrow, D., Wright, R. O., Nie, H., and Hu, H. (2007). Lead levels and ischemic heart disease in a prospective study of middle-aged and elderly men: the VA Normative Aging Study. *Environ Health Perspect* **115**, 871-875.

Jhun, H. J., Kim, H., and Paek, D. M. (2005). The association between blood metal concentrations and heart rate variability: a cross-sectional study. *Int Arch Occup Environ Health* **78**, 243-247.
Kaewboonchoo, O., Morioka, I., Saleekul, S., Miyai, N., Chaikittiporn, C., and Kawai, T. (2010). Blood lead level and cardiovascular risk factors among bus drivers in Bangkok, Thailand. *Ind Health* **48**, 61-65.
Kaewboonchoo, O., Saleekul, S., Powwattana, A., and Kawai, T. (2007). Blood lead level and blood pressure of bus drivers in Bangkok, Thailand. *Ind Health* **45**, 590-594.
Kim, K. R., Lee, S. W., Paik, N. W., and Choi, K. (2008). Low-level lead exposure among South Korean lead workers, and estimates of associated risk of cardiovascular diseases. *J Occup Environ Hyg* **5**, 399-416.
Korrick, S. A., Hunter, D. J., Rotnitzky, A., Hu, H., and Speizer, F. E. (1999). Lead and hypertension in a sample of middle-aged women. *Am J Public Health* **89**, 330-335.
Kromhout, D., Wibowo, A. A., Herber, R. F., Dalderup, L. M., Heerdink, H., de Lezenne Coulander, C., and Zielhuis, R. L. (1985). Trace metals and coronary heart disease risk indicators in 152 elderly men (the Zutphen Study). *Am J Epidemiol* **122**, 378-385.
Lakatta, E. G., and Levy, D. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. *Circ* **107**, 139-146.
Law, M. R., Morris, J. K., and Wald, N. J. (2009). Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ* **338**, b1665.
Lin, J. L., Lin-Tan, D. T., Hsu, C. W., Yen, T. H., Chen, K. H., Hsu, H. H., Ho, T. C., and Hsu, K. H. (2011). Association of blood lead levels with mortality in patients on maintenance hemodialysis. *Am J Med* **124**, 350-358.
Lockett, C. J., and Arbuckle, D. (1987). Lead, ferritin, zinc, and hypertension. *Bull Environ Contam Toxicol* **38**, 975-980.
Magri, J., Sammut, M., and Savona-Ventura, C. (2003). Lead and other metals in gestational hypertension. *Int J Gynaecol Obstet* **83**, 29-36.
Martin, D., Glass, T. A., Bandeen-Roche, K., Todd, A. C., Shi, W., and Schwartz, B. S. (2006). Association of blood lead and tibia lead with blood pressure and hypertension in a community sample of older adults. *Am J Epidemiol* **163**, 467-478.
Menditto, A., Morisi, G., Spagnolo, A., and Menotti, A. (1994). Association of blood lead to blood pressure in men aged 55 to 75 years: effect of selected social and biochemical confounders. NFR Study Group. *Environ Health Perspect* **102 Suppl 9**, 107-111.
Menke, A., Muntner, P., Batuman, V., Silbergeld, E. K., and Guallar, E. (2006). Blood lead below 0.48 micromol/L (10 microg/dL) and mortality among US adults. *Circ* **114**, 1388-1394.
Moller, L., and Kristensen, T. S. (1992). Blood lead as a cardiovascular risk factor. *Am J Epidemiol* **136**, 1091-1100.
Morris, C., McCarron, D. A., and Bennett, W. M. (1990). Low-level lead exposure, blood pressure, and calcium metabolism. *Am J Kidney Dis* **15**, 568-574.
Muntner, P., Menke, A., DeSalvo, K. B., Rabito, F. A., and Batuman, V. (2005). Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys. *Arch Intern Med* **165**, 2155-2161.
Nash, D., Magder, L., Lustberg, M., Sherwin, R. W., Rubin, R. J., Kaufmann, R. B., and Silbergeld, E. K. (2003). Blood lead, blood pressure, and hypertension in perimenopausal and postmenopausal women. *Jama* **289**, 1523-1532.
Navas-Acien, A., Schwartz, B. S., Rothenberg, S. J., Hu, H., Silbergeld, E. K., and Guallar, E. (2008). Bone lead levels and blood pressure endpoints: a meta-analysis. *Epidemiology* **19**, 496-504.
Navas-Acien, A., Selvin, E., Sharrett, A. R., Calderon-Aranda, E., Silbergeld, E., and Guallar, E. (2004). Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circ* **109**, 3196-3201.
Nawrot, T. S., Thijs, L., Den Hond, E. M., Roels, H. A., and Staessen, J. A. (2002). An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis. *J Hum Hypertens* **16**, 123-131.
Nordberg, M., Winblad, B., Fratiglioni, L., and Basun, H. (2000). Lead concentrations in elderly urban people related to blood pressure and mental performance: results from a population-based study. *Am J Ind Med* **38**, 290-294.
Orssaud, G., Claude, J. R., Moreau, T., Lellouch, J., Juguet, B., and Festy, B. (1985). Blood lead concentration and blood pressure. *Br Med J (Clin Res Ed)* **290**, 244.

Park, S. K., Hu, H., Wright, R. O., Schwartz, J., Cheng, Y., Sparrow, D., Vokonas, P. S., and Weisskopf, M. G. (2009a). Iron metabolism genes, low-level lead exposure, and QT interval. *Environ Health Perspect* **117**, 80-85.

Park, S. K., Mukherjee, B., Xia, X., Sparrow, D., Weisskopf, M. G., Nie, H., and Hu, H. (2009b). Bone lead level prediction models and their application to examine the relationship of lead exposure and hypertension in the Third National Health and Nutrition Examination Survey. *J Occup Environ Med* **51**, 1422-1436.

Park, S. K., O'Neill, M. S., Vokonas, P. S., Sparrow, D., Wright, R. O., Coull, B., Nie, H., Hu, H., and Schwartz, J. (2008). Air pollution and heart rate variability: effect modification by chronic lead exposure. *Epidemiology* **19**, 111-120.

Park, S. K., Schwartz, J., Weisskopf, M., Sparrow, D., Vokonas, P. S., Wright, R. O., Coull, B., Nie, H., and Hu, H. (2006). Low-level lead exposure, metabolic syndrome, and heart rate variability: the VA Normative Aging Study. *Environ Health Perspect* **114**, 1718-1724.

Perlstein, T., Weuve, J., Schwartz, J., Sparrow, D., Wright, R., Litonjua, A., Nie, H., and Hu, H. (2007). Cumulative community-level lead exposure and pulse pressure: the normative aging study. *Environ Health Perspect* **115**, 1696-1700.

Peters, J. L., Kubzansky, L., McNeely, E., Schwartz, J., Spiro, A., 3rd, Sparrow, D., Wright, R. O., Nie, H., and Hu, H. (2007). Stress as a potential modifier of the impact of lead levels on blood pressure: the normative aging study. *Environ Health Perspect* **115**, 1154-1159.

Pizent, A., Jurasovie, J., and Telisman, S. (2001). Blood pressure in relation to dietary calcium intake, alcohol consumption, blood lead, and blood cadmium in female nonsmokers. *J Trace Elem Med Biol* **15**, 123-130.

Pocock, S. J., Shaper, A. G., Ashby, D., Delves, H. T., and Clayton, B. E. (1988). The relationship between blood lead, blood pressure, stroke, and heart attacks in middle-aged British men. *Environ Health Perspect* **78**, 23-30.

Rabinowitz, M., Bellinger, D., Leviton, A., Needleman, H., and Schoenbaum, S. (1987). Pregnancy hypertension, blood pressure during labor, and blood lead levels. *Hypertension* **10**, 447-451.

Rothenberg, S. J., Khan, F., Manalo, M., Jiang, J., Cuellar, R., Reyes, S., Acosta, S., Jauregui, M., Diaz, M., Sanchez, M., Todd, A. C., and Johnson, C. (2000). Maternal bone lead contribution to blood lead during and after pregnancy. *Environ Res* **82**, 81-90.

Rothenberg, S. J., Kondrashov, V., Manalo, M., Jiang, J., Cuellar, R., Garcia, M., Reynoso, B., Reyes, S., Diaz, M., and Todd, A. C. (2002). Increases in hypertension and blood pressure during pregnancy with increased bone lead levels. *Am J Epidemiol* **156**, 1079-1087.

Rothenberg, S. J., Manalo, M., Jiang, J., Cuellar, R., Reyes, S., Sanchez, M., Diaz, M., Khan, F., Aguilar, A., Reynoso, B., Juaregui, M., Acosta, S., and Johnson, C. (1999). Blood lead level and blood pressure during pregnancy in South Central Los Angeles. *Arch Environ Health* **54**, 382-389.

Schober, S. E., Mirel, L. B., Graubard, B. I., Brody, D. J., and Flegal, K. M. (2006). Blood lead levels and death from all causes, cardiovascular disease, and cancer: results from the NHANES III mortality study. *Environ Health Perspect* **114**, 1538-1541.

Schuhmacher, M., Bosque, M. A., Domingo, J. L., and Corbella, J. (1994). Effects of chronic lead and cadmium exposure on blood pressure in occupationally exposed workers. *Biol Trace Elem Res* **41**, 269-278.

Schwartz, B. S., and Stewart, W. F. (2000). Different associations of blood lead, meso 2,3-dimercaptosuccinic acid (DMSA)-chelatable lead, and tibial lead levels with blood pressure in 543 former organolead manufacturing workers. *Arch Environ Health* **55**, 85-92.

Schwartz, J. (1991). Lead, blood pressure, and cardiovascular disease in men and women. *Environ Health Perspect* **91**, 71-75.

Schwartz, J. (1995). Lead, blood pressure, and cardiovascular disease in men. *Arch Environ Health* **50**, 31-37.

Scinicariello, F., Abadin, H. G., and Edward Murray, H. (2011). Association of low-level blood lead and blood pressure in NHANES 1999-2006. *Environ Res*.

Scinicariello, F., Yesupriya, A., Chang, M. H., and Fowler, B. A. (2010). Modification by ALAD of the association between blood lead and blood pressure in the U.S. population: results from the Third National Health and Nutrition Examination Survey. *Environ Health Perspect* **118**, 259-264.

Sharp, D. S., Benowitz, N. L., Osterloh, J. D., Becker, C. E., Smith, A. H., and Syme, S. L. (1990). Influence of race, tobacco use, and caffeine use on the relation between blood pressure and blood lead concentration. *Am J Epidemiol* **131**, 845-854.

Silbergeld, E. K., Schwartz, J., and Mahaffey, K. (1988). Lead and osteoporosis: mobilization of lead from bone in postmenopausal women. *Environ Res* **47**, 79-94.

Sirivarasai, J., Kaojarern, S., Wananukul, W., Deechakwan, W., and Srisomerarn, P. (2004). Non-occupational lead and cadmium exposure and blood pressure in Thai men. *Asia Pac J Public Health* **16**, 133-137.

Sowers, M., Jannausch, M., Scholl, T., Li, W., Kemp, F. W., and Bogden, J. D. (2002). Blood lead concentrations and pregnancy outcomes. *Arch Environ Health* **57**, 489-495.

Staessen, J., Yeoman, W. B., Fletcher, A. E., Markowe, H. L., Marmot, M. G., Rose, G., Semmence, A., Shipley, M. J., and Bulpitt, C. J. (1990). Blood lead concentration, renal function, and blood pressure in London civil servants. *Br J Ind Med* **47**, 442-447.

Staessen, J. A., Bulpitt, C. J., Fagard, R., Lauwerys, R. R., Roels, H., Thijs, L., and Amery, A. (1994). Hypertension caused by low-level lead exposure: myth or fact? *J Cardiovasc Risk* **1**, 87-97.

Staessen, J. A., Roels, H., and Fagard, R. (1996). Lead exposure and conventional and ambulatory blood pressure: a prospective population study. PheeCad Investigators. *Jama* **275**, 1563-1570.

Symanski, E., and Hertz-Picciotto, I. (1995). Blood lead levels in relation to menopause, smoking, and pregnancy history. *Am J Epidemiol* **141**, 1047-1058.

Tsao, D. A., Yu, H. S., Cheng, J. T., Ho, C. K., and Chang, H. R. (2000). The change of beta-adrenergic system in lead-induced hypertension. *Toxicol Appl Pharmacol* **164**, 127-133.

U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.

Vigeh, M., Yokoyama, K., Mazaheri, M., Beheshti, S., Ghazizadeh, S., Sakai, T., Morita, Y., Kitamura, F., and Araki, S. (2004). Relationship between increased blood lead and pregnancy hypertension in women without occupational lead exposure in Tehran, Iran. *Arch Environ Health* **59**, 70-75.

Weisskopf, M. G., Jain, N., Nie, H., Sparrow, D., Vokonas, P., Schwartz, J., and Hu, H. (2009). A prospective study of bone lead concentration and death from all causes, cardiovascular diseases, and cancer in the Department of Veterans Affairs Normative Aging Study. *Circ* **120**, 1056-1064.

Wells, E. M., Navas-Acien, A., Herbstman, J. B., Apelberg, B. J., Silbergeld, E. K., Caldwell, K. L., Jones, R. L., Halden, R. U., Witter, F. R., and Goldman, L. R. (2011). Low Level Lead Exposure and Elevations in Blood Pressure During Pregnancy. *Environ Health Perspect* **119**, 664-669.

Whelton, P. K., He, J., Appel, L. J., Cutler, J. A., Havas, S., Kotchen, T. A., Roccella, E. J., Stout, R., Vallbona, C., Winston, M. C., and Karimbakas, J. (2002). Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *JAMA* **288**, 1882-1888.

Wolf, C., Wallnofer, A., Waldhor, T., Vutuc, C., Meisinger, V., and Rudiger, H. W. (1995). Effect of lead on blood pressure in occupationally nonexposed men. *Am J Ind Med* **27**, 897-903.

Yazbeck, C., Thiebaugeorges, O., Moreau, T., Goua, V., Debotte, G., Sahuquillo, J., Forhan, A., Foliguet, B., Magnin, G., Slama, R., Charles, M. A., and Huel, G. (2009). Maternal blood lead levels and the risk of pregnancy-induced hypertension: the EDEN cohort study. *Environ Health Perspect* **117**, 1526-1530.

Zeller, I., Knoflach, M., Seubert, A., Kreutmayer, S. B., Stelzmuller, M. E., Wallnoefer, E., Blunder, S., Frotschnig, S., Messner, B., Willeit, J., Debbage, P., Wick, G., Kiechl, S., Laufer, G., and Bernhard, D. (2010). Lead contributes to arterial intimal hyperplasia through nuclear factor erythroid 2-related factor-mediated endothelial interleukin 8 synthesis and subsequent invasion of smooth muscle cells. *Arterioscler Thromb Vasc Biol* **30**, 1733-1740.

Zhang, A., Park, S. K., Wright, R. O., Weisskopf, M. G., Mukherjee, B., Nie, H., Sparrow, D., and Hu, H. (2010). HFE H63D Polymorphism as a Modifier of the Effect of Cumulative Lead Exposure on Pulse Pressure: the Normative Aging Study. *Environ Health Perspect* **118**, 1261-1266.

9.7 Renal Effects

Akesson, A., Lundh, T., Vahter, M., Bjellerup, P., Lidfeldt, J., Nerbrand, C., Samsioe, G., Stromberg, U., and Skerfving, S. (2005). Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. *Environ Health Perspect* **113**, 1627-1631.

Alinovi, R., Scotti, E., Andreoli, R., De Palma, G., Goldoni, M., Apostoli, P., and Mutti, A. (2005). [Neuroendocrine and renal effects of inorganic lead]. *G Ital Med Lav Ergon* **27 Suppl 1**, 33-38.

ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Bernard, A. M., Vyskocil, A., Roels, H., Kriz, J., Kodl, M., and Lauwerys, R. (1995). Renal effects in children living in the vicinity of a lead smelter. *Environ Res* **68**, 91-95.

Cardenas, A., Roels, H., Bernard, A. M., Barbon, R., Buchet, J. P., Lauwerys, R. R., Rosello, J., Ramis, I., Mutti, A., Franchini, I., and et al. (1993). Markers of early renal changes induced by industrial pollutants. II. Application to workers exposed to lead. *Br J Ind Med* **50**, 28-36.

Coria, C., Cabello, A., Tassara, E., Lopez, E., Rosales, H., Perez, M., Zavala, C., Munoz, P., Orellana, G., Inostroza, M. I., Contreras, L., and Kirsten, L. (2009). [Long term consequences among children exposed to lead poisoning]. *Revista medica de Chile* **137**, 1037-1044.

de Burbure, C., Buchet, J. P., Bernard, A., Leroyer, A., Nisse, C., Haguenoer, J. M., Bergamaschi, E., and Mutti, A. (2003). Biomarkers of renal effects in children and adults with low environmental exposure to heavy metals. *J Toxicol Environ Health A* **66**, 783-798.

de Burbure, C., Buchet, J. P., Leroyer, A., Nisse, C., Haguenoer, J. M., Mutti, A., Smerhovsky, Z., Cikrt, M., Trzcinka-Ochocka, M., Razniewska, G., Jakubowski, M., and Bernard, A. (2006). Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. *Environ Health Perspect* **114**, 584-590.

Devarajan, P. (2010). The use of targeted biomarkers for chronic kidney disease. *Advances in chronic kidney disease* **17**, 469-479.

Fadrowski, J. J., Navas-Acien, A., Tellez-Plaza, M., Guallar, E., Weaver, V. M., and Furth, S. L. (2010). Blood lead level and kidney function in US adolescents: The Third National Health and Nutrition Examination Survey. *Arch Intern Med* **170**, 75-82.

Fels, L. M., Wunsch, M., Baranowski, J., Norska-Borowka, I., Price, R. G., Taylor, S. A., Patel, S., De Broe, M., Elsevier, M. M., Lauwerys, R., Roels, H., Bernard, A., Mutti, A., Gelpi, E., Rosello, J., and Stolte, H. (1998). Adverse effects of chronic low level lead exposure on kidney function--a risk group study in children. *Nephrol Dial Transplant* **13**, 2248-2256.

Garcon, G., Leleu, B., Marez, T., Zerimech, F., Haguenoer, J. M., Furon, D., and Shirali, P. (2007). Biomonitoring of the adverse effects induced by the chronic exposure to lead and cadmium on kidney function: usefulness of alpha-glutathione S-transferase. *Sci Total Environ* **377**, 165-172.

Hu, H. (1991). A 50-year follow-up of childhood plumbism. Hypertension, renal function, and hemoglobin levels among survivors. *Am J Dis Child* **145**, 681-687.

Inglis, J. A., Henderson, D. A., and Emmerson, B. T. (1978). The pathology and pathogenesis of chronic lead nephropathy occurring in Queensland. *J Pathol* **124**, 65-76.

Khalil-Manesh, F., Gonick, H. C., Cohen, A. H., Alinovi, R., Bergamaschi, E., Mutti, A., and Rosen, V. J. (1992). Experimental model of lead nephropathy. I. Continuous high-dose lead administration. *Kidney Int* **41**, 1192-1203.

Khan, D. A., Qayyum, S., Saleem, S., Ansari, W. M., and Khan, F. A. (2010). Lead exposure and its adverse health effects among occupational worker's children. *Toxicol Ind Health* **26**, 497-504.

Khan, D. A., Qayyum, S., Saleem, S., and Khan, F. A. (2008). Lead-induced oxidative stress adversely affects health of the occupational workers. *Toxicol Ind Health* **24**, 611-618.

Kim, R., Rotnitsky, A., Sparrow, D., Weiss, S., Wager, C., and Hu, H. (1996). A longitudinal study of low-level lead exposure and impairment of renal function. The Normative Aging Study. *Jama* **275**, 1177-1181.

Lai, L. H., Chou, S. Y., Wu, F. Y., Chen, J. J., and Kuo, H. W. (2008). Renal dysfunction and hyperuricemia with low blood lead levels and ethnicity in community-based study. *Sci Total Environ* **401**, 39-43.

Levey, A. S., Bosch, J. P., Lewis, J. B., Greene, T., Rogers, N., Roth, D. C. I. N. A. I. M. O., and author reply, P. (1999). A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* **130**, 461-470.

Lin, J. L., Lin-Tan, D. T., Hsu, K. H., and Yu, C. C. (2003). Environmental lead exposure and progression of chronic renal diseases in patients without diabetes. *N Engl J Med* **348**, 277-286.

Lin, J. L., Lin-Tan, D. T., Li, Y. J., Chen, K. H., and Huang, Y. L. (2006a). Low-level environmental exposure to lead and progressive chronic kidney diseases. *Am J Med* **119**, 707 e701-709.

Lin, J. L., Lin-Tan, D. T., Yu, C. C., Li, Y. J., Huang, Y. Y., and Li, K. L. (2006b). Environmental exposure to lead and progressive diabetic nephropathy in patients with type II diabetes. *Kidney Int* **69**, 2049-2056.

Lin, T., and Tai-Yi, J. (2007). Benchmark dose approach for renal dysfunction in workers exposed to lead. *Environ Toxicol* **22**, 229-233.

Moel, D. I., and Sachs, H. K. (1992). Renal function 17 to 23 years after chelation therapy for childhood plumbism. *Kidney Int* **42**, 1226-1231.
Mortada, W. I., Sobh, M. A., and El-Defrawy, M. M. (2004). The exposure to cadmium, lead and mercury from smoking and its impact on renal integrity. *Med Sci Monit* **10**, CR112-116.
Muntner, P., He, J., Vupputuri, S., Coresh, J., and Batuman, V. (2003). Blood lead and chronic kidney disease in the general United States population: results from NHANES III. *Kidney Int* **63**, 1044-1050.
Muntner, P., Menke, A., DeSalvo, K. B., Rabito, F. A., and Batuman, V. (2005). Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys. *Arch Intern Med* **165**, 2155-2161.
Navas-Acien, A., Tellez-Plaza, M., Guallar, E., Muntner, P., Silbergeld, E., Jaar, B., and Weaver, V. (2009). Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. *Am J Epidemiol* **170**, 1156-1164.
Patil, A. J., Bhagwat, V. R., Patil, J. A., Dongre, N. N., Ambekar, J. G., and Das, K. K. (2007). Occupational lead exposure in battery manufacturing workers, silver jewelry workers, and spray painters in western Maharashtra (India): effect on liver and kidney function. *J Basic Clin Physiol Pharmacol* **18**, 87-100.
Payton, M., Hu, H., Sparrow, D., and Weiss, S. T. (1994). Low-level lead exposure and renal function in the Normative Aging Study. *Am J Epidemiol* **140**, 821-829.
Pocock, S. J., Shaper, A. G., Ashby, D., Delves, T., and Whitehead, T. P. (1984). Blood lead concentration, blood pressure, and renal function. *Br Med J (Clin Res Ed)* **289**, 872-874.
Rose, B. D., and Post, T. W. (2011). Prostaglandins and the kidney. *In: UpToDate, Basow, DS (Eds), UpToDate, Waltham, MA*.
Staessen, J., Yeoman, W. B., Fletcher, A. E., Markowe, H. L., Marmot, M. G., Rose, G., Semmence, A., Shipley, M. J., and Bulpitt, C. J. (1990). Blood lead concentration, renal function, and blood pressure in London civil servants. *Br J Ind Med* **47**, 442-447.
Staessen, J. A., Lauwerys, R. R., Buchet, J. P., Bulpitt, C. J., Rondia, D., Vanrenterghem, Y., and Amery, A. (1992). Impairment of renal function with increasing blood lead concentrations in the general population. The Cadmibel Study Group. *N Engl J Med* **327**, 151-156.
Staessen, J. A., Nawrot, T., Hond, E. D., Thijs, L., Fagard, R., Hoppenbrouwers, K., Koppen, G., Nelen, V., Schoeters, G., Vanderschueren, D., Van Hecke, E., Verschaeve, L., Vlietinck, R., and Roels, H. A. (2001). Renal function, cytogenetic measurements, and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers. *Lancet* **357**, 1660-1669.
Sun, Y., Sun, D., Zhou, Z., Zhu, G., Lei, L., Zhang, H., Chang, X., and Jin, T. (2008). Estimation of benchmark dose for bone damage and renal dysfunction in a Chinese male population occupationally exposed to lead. *Ann Occup Hyg* **52**, 527-533.
Tesch, G. H. (2010). Review: Serum and urine biomarkers of kidney disease: A pathophysiological perspective. *Nephrology (Carlton, Vic.)* **15**, 609-616.
Tsaih, S. W., Korrick, S., Schwartz, J., Amarasiriwardena, C., Aro, A., Sparrow, D., and Hu, H. (2004). Lead, diabetes, hypertension, and renal function: the normative aging study. *Environ Health Perspect* **112**, 1178-1182.
U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.
U.S. EPA (2011). Integrated Science Assessment for Lead (First External Review Draft). Office of Research and Development, National Center for Environmental Assessment-RTP Division, Research Triangle Park, NC.
Verberk, M. M., Willems, T. E., Verplanke, A. J., and De Wolff, F. A. (1996). Environmental lead and renal effects in children. *Arch Environ Health* **51**, 83-87.
Weaver, V., and Jaar, B. (2010). UpToDate: Lead nephropathy and lead-related nephrotoxicity (http://www.uptodate.com/contents/lead-nephropathy-and-lead-related-nephrotoxicity). *UpToDate, Inc.*
Weaver, V. M., Griswold, M., Todd, A. C., Jaar, B. G., Ahn, K. D., Thompson, C. B., and Lee, B. K. (2009). Longitudinal associations between lead dose and renal function in lead workers. *Environ Res* **109**, 101-117.
Weaver, V. M., Lee, B. K., Ahn, K. D., Lee, G. S., Todd, A. C., Stewart, W. F., Wen, J., Simon, D. J., Parsons, P. J., and Schwartz, B. S. (2003). Associations of lead biomarkers with renal function in Korean lead workers. *Occup Environ Med* **60**, 551-562.

Weaver, V. M., Lee, B. K., Todd, A. C., Jaar, B. G., Ahn, K. D., Wen, J., Shi, W., Parsons, P. J., and Schwartz, B. S. (2005). Associations of patella lead and other lead biomarkers with renal function in lead workers. *J Occup Environ Med* **47**, 235-243.

Wu, M. T., Kelsey, K., Schwartz, J., Sparrow, D., Weiss, S., and Hu, H. (2003). A delta-aminolevulinic acid dehydratase (ALAD) polymorphism may modify the relationship of low-level lead exposure to uricemia and renal function: the normative aging study. *Environ Health Perspect* **111**, 335-341.

Yu, C. C., Lin, J. L., and Lin-Tan, D. T. (2004). Environmental exposure to lead and progression of chronic renal diseases: a four-year prospective longitudinal study. *J Am Soc Nephrol* **15**, 1016-1022.

9.8 Reproductive and Developmental Effects

Abdelouahab, N., Mergler, D., Takser, L., Vanier, C., St-Jean, M., Baldwin, M., Spear, P. A., and Chan, H. M. (2008). Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of Quebec (Canada). *Environ Res* **107**, 380-392.

Al-Hakkak, Z. S., Hamamy, H. A., Murad, A. M., and Hussain, A. F. (1986). Chromosome aberrations in workers at a storage battery plant in Iraq. *Mutat Res* **171**, 53-60.

Al-Saleh, I., Coskun, S., Mashhour, A., Shinwari, N., El-Doush, I., Billedo, G., Jaroudi, K., Al-Shahrani, A., Al-Kabra, M., and El Din Mohamed, G. (2008a). Exposure to heavy metals (lead, cadmium and mercury) and its effect on the outcome of in-vitro fertilization treatment. *Int J Hyg Environ Health* **211**, 560-579.

Al-Saleh, I., Shinwari, N., Nester, M., Mashhour, A., Moncari, L., El Din Mohamed, G., and Rabah, A. (2008b). Longitudinal study of prenatal and postnatal lead exposure and early cognitive development in Al-Kharj, Saudi Arabia: a preliminary results of cord blood lead levels. *J Trop Pediatr* **54**, 300-307.

Alexander, B. H., Checkoway, H., Faustman, E. M., van Netten, C., Muller, C. H., and Ewers, T. G. (1998). Contrasting associations of blood and semen lead concentrations with semen quality among lead smelter workers. *Am J Ind Med* **34**, 464-469.

Alexander, B. H., Checkoway, H., Van Netten, C., Kaufman, J. D., Vaughan, T. L., Mueller, B. A., and Faustman, E. M. (1996a). Paternal Occupational Lead Exposure and Pregnancy Outcome. *Int J Occup Environ Health* **2**, 280-285.

Alexander, B. H., Checkoway, H., van Netten, C., Muller, C. H., Ewers, T. G., Kaufman, J. D., Mueller, B. A., Vaughan, T. L., and Faustman, E. M. (1996b). Semen quality of men employed at a lead smelter. *Occup Environ Med* **53**, 411-416.

Angell, N. F., and Lavery, J. P. (1982). The relationship of blood lead levels to obstetric outcome. *Am J Obstet Gynecol* **142**, 40-46.

Apostoli, P., Bellini, A., Porru, S., and Bisanti, L. (2000). The effect of lead on male fertility: a time to pregnancy (TTP) study. *Am J Ind Med* **38**, 310-315.

Aschengrau, A., Zierler, S., and Cohen, A. (1993). Quality of community drinking water and the occurrence of late adverse pregnancy outcomes. *Arch Environ Health* **48**, 105-113.

Assennato, G., Paci, C., Baser, M. E., Molinini, R., Candela, R. G., Altamura, B. M., and Giorgino, R. (1986). Sperm count suppression without endocrine dysfunction in lead-exposed men. *Arch Environ Health* **41**, 387-390.

Assennato, G., Paci, C., Baser, M. E., Molinini, R., Candela, R. G., Altamura, B. M., and Giorgino, R. (1987). Sperm count suppression without endocrine dysfunction in lead-exposed men. *Arch Environ Health* **42**, 124-127.

ATSDR (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Baghurst, P. A., Robertson, E. F., Oldfield, R. K., King, B. M., McMichael, A. J., Vimpani, G. V., and Wigg, N. R. (1991). Lead in the placenta, membranes, and umbilical cord in relation to pregnancy outcome in a lead-smelter community. *Environ Health Perspect* **90**, 315-320.

Ballew, C., Khan, L. K., Kaufmann, R., Mokdad, A., Miller, D. T., and Gunter, E. W. (1999). Blood lead concentration and children's anthropometric dimensions in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. *J Pediatr* **134**, 623-630.

Beckman, L., and Nordstrom, S. (1982). Occupational and environmental risks in and around a smelter in northern Sweden. IX. Fetal mortality among wives of smelter workers. *Hereditas* **97**, 1-7.

Bellinger, D., Leviton, A., Rabinowitz, M., Allred, E., Needleman, H., and Schoenbaum, S. (1991). Weight gain and maturity in fetuses exposed to low levels of lead. *Environ Res* **54**, 151-158.

Benoff, S., Centola, G. M., Millan, C., Napolitano, B., Marmar, J. L., and Hurley, I. R. (2003a). Increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. *Hum Reprod* **18**, 374-383.

Benoff, S., Hurley, I. R., Millan, C., Napolitano, B., and Centola, G. M. (2003b). Seminal lead concentrations negatively affect outcomes of artificial insemination. *Fertil Steril* **80**, 517-525.

Bloom, M. S., Louis, G. M., Sundaram, R., Kostyniak, P. J., and Jain, J. (2011a). Associations between blood metals and fecundity among women residing in New York State. *Reprod Toxicol* **31**, 158-163.

Bloom, M. S., Parsons, P. J., Kim, D., Steuerwald, A. J., Vaccari, S., Cheng, G., and Fujimoto, V. Y. (2011b). Toxic trace metals and embryo quality indicators during in vitro fertilization (IVF). *Reprod Toxicol* **31**, 164-170.

Bloom, M. S., Parsons, P. J., Steuerwald, A. J., Schisterman, E. F., Browne, R. W., Kim, K., Coccaro, G. A., Conti, G. C., Narayan, N., and Fujimoto, V. Y. (2010). Toxic trace metals and human oocytes during in vitro fertilization (IVF). *Reprod Toxicol* **29**, 298-305.

Bonde, J. P., Joffe, M., Apostoli, P., Dale, A., Kiss, P., Spano, M., Caruso, F., Giwercman, A., Bisanti, L., Porru, S., Vanhoorne, M., Comhaire, F., and Zschiesche, W. (2002). Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. *Occup Environ Med* **59**, 234-242.

Bonde, J. P., and Kolstad, H. (1997). Fertility of Danish battery workers exposed to lead. *Int J Epidemiol* **26**, 1281-1288.

Borja-Aburto, V. H., Hertz-Picciotto, I., Rojas Lopez, M., Farias, P., Rios, C., and Blanco, J. (1999). Blood lead levels measured prospectively and risk of spontaneous abortion. *Am J Epidemiol* **150**, 590-597.

Bornschein, R. L., Grote, J., Mitchell, T., Succop, P., Dietrich, K. N., Krafft, K. M., and Hammond, P. B. (1989). Effects of prenatal lead exposure on infant size at birth. In Lead Exposure and Child Development: An International Assessment M. A. Smith, L. D. Grante and A. I. Sors, eds., pp. 307-319. Kluwer Publishers, Lancaster, UK.

Bound, J. P., Harvey, P. W., Francis, B. J., Awwad, F., and Gatrell, A. C. (1997). Involvement of deprivation and environmental lead in neural tube defects: a matched case-control study. *Arch Dis Child* **76**, 107-112.

Braunstein, G. D., Dahlgren, J., and Loriaux, D. L. (1978). Hypogonadism in chronically lead-poisoned men. *Infertility* **1**, 33-51.

Brender, J., Suarez, L., Hendricks, K., Baetz, R. A., and Larsen, R. (2002). Parental occupation and neural tube defect-affected pregnancies among Mexican Americans. *J Occup Environ Med* **44**, 650-656.

Brender, J. D., Suarez, L., Felkner, M., Gilani, Z., Stinchcomb, D., Moody, K., Henry, J., and Hendricks, K. (2006). Maternal exposure to arsenic, cadmium, lead, and mercury and neural tube defects in offspring. *Environ Res* **101**, 132-139.

Cantonwine, D., Hu, H., Sanchez, B. N., Lamadrid-Figueroa, H., Smith, D., Ettinger, A. S., Mercado-Garcia, A., Hernandez-Avila, M., Wright, R. O., and Tellez-Rojo, M. M. (2010a). Critical windows of fetal lead exposure: adverse impacts on length of gestation and risk of premature delivery. *J Occup Environ Med* **52**, 1106-1111.

Cantonwine, D., Hu, H., Tellez-Rojo, M. M., Sanchez, B. N., Lamadrid-Figueroa, H., Ettinger, A. S., Mercado-Garcia, A., Hernandez-Avila, M., and Wright, R. O. (2010b). HFE gene variants modify the association between maternal lead burden and infant birthweight: a prospective birth cohort study in Mexico City, Mexico. *Environ Health* **9**, 43.

Chang, S. H., Cheng, B. H., Lee, S. L., Chuang, H. Y., Yang, C. Y., Sung, F. C., and Wu, T. N. (2006). Low blood lead concentration in association with infertility in women. *Environ Res* **101**, 380-386.

Chen, P. C., Pan, I. J., and Wang, J. D. (2006). Parental exposure to lead and small for gestational age births. *Am J Ind Med* **49**, 417-422.

Chia, S. E., Ong, C. N., Lee, S. T., and Tsakok, F. H. (1992). Blood concentrations of lead, cadmium, mercury, zinc, and copper and human semen parameters. *Arch Androl* **29**, 177-183.

Chowdhury, A. R., Chinoy, N. J., Gautam, A. K., Rao, R. V., Parikh, D. J., Shah, G. M., Highland, H. N., Patel, K. G., and Chatterjee, B. B. (1986). Effect of lead on human semen. *Adv Contracept Deliv Syst* **2**, 208-210.

Coste, J., Mandereau, L., Pessione, F., Bregu, M., Faye, C., Hemon, D., and Spira, A. (1991). Lead-exposed workmen and fertility: a cohort study on 354 subjects. *Eur J Epidemiol* **7**, 154-158.

Croen, L. A., Shaw, G. M., Sanbonmatsu, L., Selvin, S., and Buffler, P. A. (1997). Maternal residential proximity to hazardous waste sites and risk for selected congenital malformations. *Epidemiology* **8**, 347-354.

Cullen, M. R., Kayne, R. D., and Robins, J. M. (1984). Endocrine and reproductive dysfunction in men associated with occupational inorganic lead intoxication. *Arch Environ Health* **39**, 431-440.

Dawson, E. B., Evans, D. R., Harris, W. A., and Van Hook, J. W. (1999). Amniotic fluid B12, calcium, and lead levels associated with neural tube defects. *Am J Perinatol* **16**, 373-378.

De Rosa, M., Zarrilli, S., Paesano, L., Carbone, U., Boggia, B., Petretta, M., Maisto, A., Cimmino, F., Puca, G., Colao, A., and Lombardi, G. (2003). Traffic pollutants affect fertility in men. *Hum Reprod* **18**, 1055-1061.

Denham, M., Schell, L. M., Deane, G., Gallo, M. V., Ravenscroft, J., and DeCaprio, A. P. (2005). Relationship of lead, mercury, mirex, dichlorodiphenyldichloroethylene, hexachlorobenzene, and polychlorinated biphenyls to timing of menarche among Akwesasne Mohawk girls. *Pediatrics* **115**, e127-134.

Dietrich, K. N., Krafft, K. M., Shukla, R., Bornschein, R. L., and Succop, P. A. (1987). The neurobehavioral effects of early lead exposure. *Monogr Am Assoc Ment Defic*, 71-95.

Driscoll, R. J. (1998). Health hazard evaluation report 93-1035-2686, Section 2: Epidemiologic study of adverse reproductive outcomes among women in the U.S. Forest Service. U.S. Department of Agriculture, U.S. Forest Service, Washington DC.

Elwood, J. M., and Coldman, A. J. (1981). Water composition in the etiology of anencephalus. *Am J Epidemiol* **113**, 681-690.

Erfurth, E. M., Gerhardsson, L., Nilsson, A., Rylander, L., Schutz, A., Skerfving, S., and Borjesson, J. (2001). Effects of lead on the endocrine system in lead smelter workers. *Arch Environ Health* **56**, 449-455.

Ernhart, C. B., Wolf, A. W., Kennard, M. J., Erhard, P., Filipovich, H. F., and Sokol, R. J. (1986). Intrauterine exposure to low levels of lead: the status of the neonate. *Arch Environ Health* **41**, 287-291.

Factor-Litvak, P., Graziano, J. H., Kline, J. K., Popovac, D., Mehmeti, A., Ahmedi, G., Shrout, P., Murphy, M. J., Gashi, E., Haxhiu, R., and et al. (1991). A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. *Int J Epidemiol* **20**, 722-728.

Factor-Litvak, P., Wasserman, G., Kline, J. K., and Graziano, J. (1999). The Yugoslavia Prospective Study of environmental lead exposure. *Environ Health Perspect* **107**, 9-15.

Fagher, U., Laudanski, T., Schutz, A., Sipowicz, M., and Akerlund, M. (1993). The relationship between cadmium and lead burdens and preterm labor. *Int J Gynaecol Obstet* **40**, 109-114.

Fahim, M. S., Fahim, Z., and Hall, D. G. (1976). Effects of subtoxic lead levels on pregnant women in the state of Missouri. *Res Commun Chem Pathol Pharmacol* **13**, 309-331.

Falcón, M., Viñas, P., and Luna, A. (2003). Placental lead and outcome of pregnancy. *Toxicology* **185**, 59-66.

Fisher-Fischbein, J., Fischbein, A., Melnick, H. D., and Bardin, C. W. (1987). Correlation between biochemical indicators of lead exposure and semen quality in a lead-poisoned firearms instructor. *Jama* **257**, 803-805.

Frisancho, A. R., and Ryan, A. S. (1991). Decreased stature associated with moderate blood lead concentrations in Mexican-American children. *Am J Clin Nutr* **54**, 516-519.

Gandley, R., Anderson, L., and Silbergeld, E. K. (1999). Lead: male-mediated effects on reproduction and development in the rat. *Environ Res* **80**, 355-363.

Gennart, J. P., Bernard, A., and Lauwerys, R. (1992a). Assessment of thyroid, testes, kidney and autonomic nervous system function in lead-exposed workers. *Int Arch Occup Environ Health* **64**, 49-57.

Gennart, J. P., Buchet, J. P., Roels, H., Ghyselen, P., Ceulemans, E., and Lauwerys, R. (1992b). Fertility of male workers exposed to cadmium, lead, or manganese. *Am J Epidemiol* **135**, 1208-1219.

Gollenberg, A. L., Hediger, M. L., Lee, P. A., Himes, J. H., and Buck Louis, G. M. (2010). Association Between Lead and Cadmium and Reproductive Hormones in Peripubertal U.S. Girls. *Environ Health Perspect* **in press**.

Gonzalez-Cossio, T., Peterson, K. E., Sanin, L. H., Fishbein, E., Palazuelos, E., Aro, A., Hernandez-Avila, M., and Hu, H. (1997). Decrease in birth weight in relation to maternal bone-lead burden. *Pediatrics* **100**, 856-862.

Gracia, C. R., Sammel, M. D., Coutifaris, C., Guzick, D. S., and Barnhart, K. T. (2005). Occupational exposures and male infertility. *Am J Epidemiol* **162**, 729-733.

Greene, T., and Ernhart, C. B. (1991). Prenatal and preschool age lead exposure: relationship with size. *Neurotoxicol Teratol* **13**, 417-427.

Gundacker, C., Frohlich, S., Graf-Rohrmeister, K., Eibenberger, B., Jessenig, V., Gicic, D., Prinz, S., Wittmann, K. J., Zeisler, H., Vallant, B., Pollak, A., and Husslein, P. (2010). Perinatal lead and mercury exposure in Austria. *Sci Total Environ* **in press**.

Gustafson, A., Hedner, P., Schutz, A., and Skerfving, S. (1989). Occupational lead exposure and pituitary function. *Int Arch Occup Environ Health* **61**, 277-281.

Hauser, R., Sergeyev, O., Korrick, S., Lee, M. M., Revich, B., Gitin, E., Burns, J. S., and Williams, P. L. (2008). Association of blood lead levels with onset of puberty in Russian boys. *Environ Health Perspect* **116**, 976-980.

Hernandez-Avila, M., Peterson, K. E., Gonzalez-Cossio, T., Sanin, L. H., Aro, A., Schnaas, L., and Hu, H. (2002). Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants. *Arch Environ Health* **57**, 482-428.

Hernandez-Avila, M., Smith, D., Meneses, F., Sanin, L. H., and Hu, H. (1998). The influence of bone and blood lead on plasma lead levels in environmentally exposed adults. *Environ Health Perspect* **106**, 473-477.

Hernandez-Ochoa, I., Garcia-Vargas, G., Lopez-Carrillo, L., Rubio-Andrade, M., Moran-Martinez, J., Cebrian, M. E., and Quintanilla-Vega, B. (2005). Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. *Reprod Toxicol* **20**, 221-228.

Hertz-Picciotto, I. (2000). The evidence that lead increases the risk for spontaneous abortion. *Am J Ind Med* **38**, 300-309.

Hsieh, S. J., Chiu, Y. W., Li, W. F., Wu, C. H., Chen, H. I., and Chuang, H. Y. (2009). Increased concentrations of serum inhibin B among male workers with long-term moderate lead exposure. *Sci Total Environ* **407**, 2603-2607.

Hsu, P. C., Chang, H. Y., Guo, Y. L., Liu, Y. C., and Shih, T. S. (2009). Effect of smoking on blood lead levels in workers and role of reactive oxygen species in lead-induced sperm chromatin DNA damage. *Fertil Steril* **91**, 1096-1103.

Huel, G., Boudene, C., and Ibrahim, M. A. (1981). Cadmium and lead content of maternal and newborn hair: relationship to parity, birth weight, and hypertension. *Arch Environ Health* **36**, 221-227.

Iavicoli, I., Carelli, G., Stanek, E. J., 3rd, Castellino, N., and Calabrese, E. J. (2004). Effects of low doses of dietary lead on puberty onset in female mice. *Reprod Toxicol* **19**, 35-41.

Ignasiak, Z., SÅawiÅ„ska, T., RoÅ»ek, K., Little, B. B., and Malina, R. M. (2006). Lead and growth status of schoolchildren living in the copper basin of south-western Poland: Differential effects on bone growth. *Ann Hum Biol* **33**, 401-414.

Iijima, K., Otake, T., Yoshinaga, J., Ikegami, M., Suzuki, E., Naruse, H., Yamanaka, T., Shibuya, N., Yasumizu, T., and Kato, N. (2007). Cadmium, lead, and selenium in cord blood and thyroid hormone status of newborns. *Biol Trace Elem Res* **119**, 10-18.

Irgens, A., Kruger, K., Skorve, A. H., and Irgens, L. M. (1998). Reproductive outcome in offspring of parents occupationally exposed to lead in Norway. *Am J Ind Med* **34**, 431-437.

Jackson, L. W., Correa-Villasenor, A., Lees, P. S., Dominici, F., Stewart, P. A., Breysse, P. N., and Matanoski, G. (2004). Parental lead exposure and total anomalous pulmonary venous return. *Birth Defects Res A Clin Mol Teratol* **70**, 185-193.

Jelliffe-Pawlowski, L. L., Miles, S. Q., Courtney, J. G., Materna, B., and Charlton, V. (2006). Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. *J Perinatol* **26**, 154-162.

Jockenhovel, F., Bals-Pratsch, M., Bertram, H. P., and Nieschlag, E. (1990). Seminal lead and copper in fertile and infertile men. *Andrologia* **22**, 503-511.

Joffe, M., Bisanti, L., Apostoli, P., Kiss, P., Dale, A., Roeleveld, N., Lindbohm, M. L., Sallmen, M., Vanhoorne, M., and Bonde, J. P. (2003). Time To Pregnancy and occupational lead exposure. *Occup Environ Med* **60**, 752-758.

Jones, E. A., Wright, J. M., Rice, G., Buckley, B. T., Magsumbol, M. S., Barr, D. B., and Williams, B. L. (2010). Metal exposures in an inner-city neonatal population. *Environ Int* **36**, 649-654.

Kafourou, A., Touloumi, G., Makropoulos, V., Loutradi, A., Papanagiotou, A., and Hatzakis, A. (1997). Effects of lead on the somatic growth of children. *Arch Environ Health* **52**, 377-383.

Kasperczyk, A., Kasperczyk, S., Horak, S., Ostalowska, A., Grucka-Mamczar, E., Romuk, E., Olejek, A., and Birkner, E. (2008). Assessment of semen function and lipid peroxidation among lead exposed men. *Toxicol Appl Pharmacol* **228**, 378-384.

Kim, R., Hu, H., Rotnitzky, A., Bellinger, D., and Needleman, H. (1995). A longitudinal study of chronic lead exposure and physical growth in Boston children. *Environ Health Perspect* **103**, 952-957.

Kiziler, A. R., Aydemir, B., Onaran, I., Alici, B., Ozkara, H., Gulyasar, T., and Akyolcu, M. C. (2007). High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. *Biol Trace Elem Res* **120**, 82-91.

Kordas, K., Ettinger, A. S., Lamadrid-Figueroa, H., Tellez-Rojo, M. M., Hernandez-Avila, M., Hu, H., and Wright, R. O. (2009). Methylenetetrahydrofolate reductase (MTHFR) C677T, A1298C and G1793A genotypes, and the relationship between maternal folate intake, tibia lead and infant size at birth. *Br J Nutr* **102**, 907-914.

Kordas, K., Lopez, P., Rosado, J. L., Garcia Vargas, G., Alatorre Rico, J., Ronquillo, D., Cebrian, M. E., and Stoltzfus, R. J. (2004). Blood lead, anemia, and short stature are independently associated with cognitive performance in Mexican school children. *J Nutr* **134**, 363-371.

Krieg, E. F., Jr. (2007). The relationships between blood lead levels and serum follicle stimulating hormone and luteinizing hormone in the third National Health and Nutrition Examination Survey. *Environ Res* **104**, 374-382.

Kristensen, P., Irgens, L. M., Daltveit, A. K., and Andersen, A. (1993). Perinatal outcome among children of men exposed to lead and organic solvents in the printing industry. *Am J Epidemiol* **137**, 134-144.

Lamadrid-Figueroa, H., Tellez-Rojo, M. M., Hernandez-Avila, M., Trejo-Valdivia, B., Solano-Gonzalez, M., Mercado-Garcia, A., Smith, D., Hu, H., and Wright, R. O. (2007). Association between the plasma/whole blood lead ratio and history of spontaneous abortion: a nested cross-sectional study. *BMC Pregnancy Childbirth* **7**, 22.

Lamb, M. R., Janevic, T., Liu, X., Cooper, T., Kline, J., and Factor-Litvak, P. (2008). Environmental lead exposure, maternal thyroid function, and childhood growth. *Environ Res* **106**, 195-202.

Lancranjan, I., Popescu, H. I., O, G. A., Klepsch, I., and Serbanescu, M. (1975). Reproductive ability of workmen occupationally exposed to lead. *Arch Environ Health* **30**, 396-401.

Lerda, D. (1992). Study of sperm characteristics in persons occupationally exposed to lead. *Am J Ind Med* **22**, 567-571.

Lin, S., Hwang, S. A., Marshall, E. G., and Marion, D. (1998). Does paternal occupational lead exposure increase the risks of low birth weight or prematurity? *Am J Epidemiol* **148**, 173-181.

Lin, S., Hwang, S. A., Marshall, E. G., Stone, R., and Chen, J. (1996). Fertility rates among lead workers and professional bus drivers: a comparative study. *Ann Epidemiol* **6**, 201-208.

Lindbohm, M. L., Hemminki, K., Bonhomme, M. G., Anttila, A., Rantala, K., Heikkila, P., and Rosenberg, M. J. (1991a). Effects of paternal occupational exposure on spontaneous abortions. *Am J Public Health* **81**, 1029-1033.

Lindbohm, M. L., Sallmen, M., Anttila, A., Taskinen, H., and Hemminki, K. (1991b). Paternal occupational lead exposure and spontaneous abortion. *Scand J Work Environ Health* **17**, 95-103.

Little, B. B., Snell, L. M., Johnston, W. L., Knoll, K. A., and Buschang, P. H. (1990). Blood lead levels and growth status of children. *Am J Hum Biol* **2**, 265-269.

Little, B. B., Spalding, S., Walsh, B., Keyes, D. C., Wainer, J., Pickens, S., Royster, M., Villanacci, J., and Gratton, T. (2009). Blood lead levels and growth status among African-American and Hispanic children in Dallas, Texas--1980 and 2002: Dallas Lead Project II. *Ann Hum Biol* **36**, 331-341.

Llanos, M. N., and Ronco, A. M. (2009). Fetal growth restriction is related to placental levels of cadmium, lead and arsenic but not with antioxidant activities. *Reprod Toxicol* **27**, 88-92.

Loiacono, N. J., Graziano, J. H., Kline, J. K., Popovac, D., Ahmedi, X., Gashi, E., Mehmeti, A., and Rajovic, B. (1992). Placental cadmium and birthweight in women living near a lead smelter. *Arch Environ Health* **47**, 250-255.

Lopez, C. M., Pineiro, A. E., Nunez, N., Avagnina, A. M., Villaamil, E. C., and Roses, O. E. (2000). Thyroid hormone changes in males exposed to lead in the Buenos Aires area (Argentina). *Pharmacol Res* **42**, 599-602.

Lorente, C., Cordier, S., Bergeret, A., De Walle, H. E., Goujard, J., Ayme, S., Knill-Jones, R., Calzolari, E., and Bianchi, F. (2000). Maternal occupational risk factors for oral clefts. Occupational Exposure and Congenital Malformation Working Group. *Scand J Work Environ Health* **26**, 137-145.

Macdonell, J. E., Campbell, H., and Stone, D. H. (2000). Lead levels in domestic water supplies and neural tube defects in Glasgow. *Arch Dis Child* **82**, 50-53.

Mahmoud, A., Kiss, P., Vanhoorne, M., De Bacquer, D., and Comhaire, F. (2005). Is inhibin B involved in the toxic effect of lead on male reproduction? *Int J Androl* **28**, 150-155.

McGregor, A., and Mason, H. (1991). The effects of occupational exposure to cadmium, lead and mercury vapour on male reproductive endocrine function. In Proceedings of the International Conference on Heavy Metals in the Environment J. Farmer, ed., Vol. Vol 1, pp. 375-378. CEP Consultants, Edinburgh, UK.

McGregor, A. J., and Mason, H. J. (1990). Chronic occupational lead exposure and testicular endocrine function. *Hum Exp Toxicol* **9**, 371-376.

McMichael, A. J., Vimpani, G. V., Robertson, E. F., Baghurst, P. A., and Clark, P. D. (1986). The Port Pirie cohort study: maternal blood lead and pregnancy outcome. *J Epidemiol Community Health* **40**, 18-25.

Meeker, J. D., Rossano, M. G., Protas, B., Diamond, M. P., Puscheck, E., Daly, D., Paneth, N., and Wirth, J. J. (2008). Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. *Environ Health Perspect* **116**, 1473-1479.

Meeker, J. D., Rossano, M. G., Protas, B., Padmanahban, V., Diamond, M. P., Puscheck, E., Daly, D., Paneth, N., and Wirth, J. J. (2010). Environmental exposure to metals and male reproductive hormones: circulating testosterone is inversely associated with blood molybdenum. *Fertil Steril* **93**, 130-140.

Mendiola, J., Moreno, J. M., Roca, M., Vergara-Juarez, N., Martinez-Garcia, M. J., Garcia-Sanchez, A., Elvira-Rendueles, B., Moreno-Grau, S., Lopez-Espin, J. J., Ten, J., Bernabeu, R., and Torres-Cantero, A. M. (2011). Relationships between heavy metal concentrations in three different body fluids and male reproductive parameters: a pilot study. *Environ Health* **10**, 6.

Min, K.-B., Min, J.-Y., Cho, S.-I., Kim, R., Kim, H., and Paek, D. (2008). Relationship between low blood lead levels and growth in children of white-collar civil servants in Korea. *Int J Hyg Environ Health* **211**, 82-87.

Min, Y. I., Correa-Villasenor, A., and Stewart, P. A. (1996). Parental occupational lead exposure and low birth weight. *Am J Ind Med* **30**, 569-578.

Moore, M. R., Goldberg, A., Pocock, S. J., Meredith, A., Stewart, I. M., MacAnespie, H., Lees, R., and Low, A. (1982). Some studies of maternal and infant lead exposure in Glasgow. *Scott Med J* **27**, 113-122.

Murphy, M. J., Graziano, J. H., Popovac, D., Kline, J. K., Mehmeti, A., Factor-Litvak, P., Ahmedi, G., Shrout, P., Rajovic, B., Nenezic, D. U., and et al. (1990). Past pregnancy outcomes among women living in the vicinity of a lead smelter in Kosovo, Yugoslavia. *Am J Public Health* **80**, 33-35.

Naha, N., Bhar, R. B., Mukherjee, A., and Chowdhury, A. R. (2005). Structural alteration of spermatozoa in the persons employed in lead acid battery factory. *Indian J Physiol Pharmacol* **49**, 153-162.

Naha, N., and Chowdhury, A. R. (2006). Inorganic lead exposure in battery and paint factory: effect on human sperm structure and functional activity. *J UOEH* **28**, 157-171.

Naha, N., and Manna, B. (2007). Mechanism of lead induced effects on human spermatozoa after occupational exposure. *Kathmandu Univ Med J (KUMJ)* **5**, 85-94.

Naicker, N., Norris, S. A., Mathee, A., Becker, P., and Richter, L. (2010). Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: findings from the Birth to Twenty cohort. *Sci Total Environ* **408**, 4949-4954.

Needleman, H. L., Rabinowitz, M., Leviton, A., Linn, S., and Schoenbaum, S. (1984). The relationship between prenatal exposure to lead and congenital anomalies. *Jama* **251**, 2956-2959.

Neuspiel, D. R., Markowitz, M., and Drucker, E. (1994). Intrauterine cocaine, lead, and nicotine exposure and fetal growth. *Am J Public Health* **84**, 1492-1495.

Ng, T. P., Goh, H. H., Ng, Y. L., Ong, H. Y., Ong, C. N., Chia, K. S., Chia, S. E., and Jeyaratnam, J. (1991). Male endocrine functions in workers with moderate exposure to lead. *Br J Ind Med* **48**, 485-491.

Noack-Fuller, G., De Beer, C., and Seibert, H. (1993). Cadmium, lead, selenium, and zinc in semen of occupationally unexposed men. *Andrologia* **25**, 7-12.

Nordstrom, S., Beckman, L., and Nordenson, I. (1978). Occupational and environmental risks in and around a smelter in northern Sweden. III. Frequencies of spontaneous abortion. *Hereditas* **88**, 51-54.

Nordstrom, S., Beckman, L., and Nordenson, I. (1979). Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous abortion among female employees and decreased birth weight in their offspring. *Hereditas* **90**, 291-296.

Odland, J. O., Nieboer, E., Romanova, N., and Thomassen, Y. (2004). Elements in placenta and pregnancy outcome in arctic and subarctic areas. *Int J Circumpolar Health* **63**, 169-187.

Odland, J. O., Nieboer, E., Romanova, N., Thomassen, Y., and Lund, E. (1999). Blood lead and cadmium and birth weight among sub-arctic and arctic populations of Norway and Russia. *Acta Obstet Gynecol Scand* **78**, 852-860.

Osman, K., Akesson, A., Berglund, M., Bremme, K., Schutz, A., Ask, K., and Vahter, M. (2000). Toxic and essential elements in placentas of Swedish women. *Clin Biochem* **33**, 131-138.

Pace, B. M., Lawrence, D. A., Behr, M. J., Parsons, P. J., and Dias, J. A. (2005). Neonatal lead exposure changes quality of sperm and number of macrophages in testes of BALB/c mice. *Toxicology* **210**, 247-256.

Patel, A. B., and Prabhu, A. S. (2009). Determinants of lead level in umbilical cord blood. *Indian Pediatr* **46**, 791-793.

Pinon-Lataillade, G., Thoreux-Manlay, A., Coffigny, H., Masse, R., and Soufir, J. C. (1995). Reproductive toxicity of chronic lead exposure in male and female mice. *Hum Exp Toxicol* **14**, 872-878.

Plechaty, M. M., Noll, B., and Sunderman, F. W., Jr. (1977). Lead concentrations in semen of healthy men without occupational exposure to lead. *Ann Clin Lab Sci* **7**, 515-518.

Rahman, A., and Hakeem, A. (2003). Blood lead levels during pregnancy and pregnancy outcome in Karachi women. *J Pak Med Assoc* **53**, 529-533.

Rajegowda, B. K., Glass, L., and Evans, H. E. (1972). Lead concentrations in the newborn infant. *J Pediatr* **80**, 116-117.

Richter, J., Hajek, Z., Pfeifer, I., and Subrt, P. (1999). Relation between concentration of lead, zinc and lysozyme in placentas of women with intrauterine foetal growth retardation. *Cent Eur J Public Health* **7**, 40-42.

Robins, J. M., Cullen, M. R., Connors, B. B., and Kayne, R. D. (1983). Depressed thyroid indexes associated with occupational exposure to inorganic lead. *Arch Intern Med* **143**, 220-224.

Robins, T. G., Bornman, M. S., Ehrlich, R. I., Cantrell, A. C., Pienaar, E., Vallabh, J., and Miller, S. (1997). Semen quality and fertility of men employed in a South African lead acid battery plant. *Am J Ind Med* **32**, 369-376.

Rodamilans, M., Osaba, M. J., To-Figueras, J., Rivera Fillat, F., Marques, J. M., Perez, P., and Corbella, J. (1988). Lead toxicity on endocrine testicular function in an occupationally exposed population. *Hum Toxicol* **7**, 125-128.

Roses, O. E., Alvarez, S., Conti, M. I., Nobile, R. A., and Villaamil, E. C. (1989). Correlation between lead and prolactin in males exposed and unexposed to lead in Buenos Aires (Argentina) area. *Bull Environ Contam Toxicol* **42**, 438-442.

Rothenberg, S. J., Schnaas-Arrieta, L., Perez-Guerrero, I. A., Perroni-Hernandez, E., Mercado-Torres, L., Gomez-Ruiz, C., and Zea, F. (1993). Prenatal and postnatal blood lead level and head circumference in children to three years: preliminary results from the Mexico City Prospective Lead Study. *J Expo Anal Environ Epidemiol* **3 Suppl 1**, 165-172.

Rothenberg, S. J., Schnaas, L., Perroni, E., Hernandez, R. M., Martinez, S., and Hernandez, C. (1999). Pre- and postnatal lead effect on head circumference: a case for critical periods. *Neurotoxicol Teratol* **21**, 1-11.

Saaranen, M., Suistomaa, U., Kantola, M., Saarikoski, S., and Vanha-Perttula, T. (1987). Lead, magnesium, selenium and zinc in human seminal fluid: comparison with semen parameters and fertility. *Hum Reprod* **2**, 475-479.

Sallmen, M., Anttila, A., Lindbohm, M. L., Kyyronen, P., Taskinen, H., and Hemminki, K. (1995). Time to pregnancy among women occupationally exposed to lead. *J Occup Environ Med* **37**, 931-934.

Sallmen, M., Lindbohm, M. L., Anttila, A., Taskinen, H., and Hemminki, K. (1992). Paternal occupational lead exposure and congenital malformations. *J Epidemiol Community Health* **46**, 519-522.

Sallmen, M., Lindbohm, M. L., Anttila, A., Taskinen, H., and Hemminki, K. (2000a). Time to pregnancy among the wives of men occupationally exposed to lead. *Epidemiology* **11**, 141-147.

Sallmen, M., Lindbohm, M. L., and Nurminen, M. (2000b). Paternal exposure to lead and infertility. *Epidemiology* **11**, 148-152.

Sanin, L. H., Gonzalez-Cossio, T., Romieu, I., Peterson, K. E., Ruiz, S., Palazuelos, E., Hernandez-Avila, M., and Hu, H. (2001). Effect of maternal lead burden on infant weight and weight gain at one month of age among breastfed infants. *Pediatrics* **107**, 1016-1023.

Satin, K. P., Neutra, R. R., Guirguis, G., and Flessel, P. (1991). Umbilical cord blood lead levels in California. *Arch Environ Health* **46**, 167-173.

Savitz, D. A., Whelan, E. A., and Kleckner, R. C. (1989). Effect of parents' occupational exposures on risk of stillbirth, preterm delivery, and small-for-gestational-age infants. *Am J Epidemiol* **129**, 1201-1218.

Schell, L. M., Denham, M., Stark, A. D., Parsons, P. J., and Schulte, E. E. (2009). Growth of infants' length, weight, head and arm circumferences in relation to low levels of blood lead measured serially. *Am J Hum Biol* **21**, 180-187.

Schumacher, C., Brodkin, C. A., Alexander, B., Cullen, M., Rainey, P. M., van Netten, C., Faustman, E., and Checkoway, H. (1998). Thyroid function in lead smelter workers: absence of subacute or cumulative effects with moderate lead burdens. *Int Arch Occup Environ Health* **71**, 453-458.

Schwartz, J., Angle, C., and Pitcher, H. (1986). Relationship between childhood blood lead levels and stature. *Pediatrics* **77**, 281-288.

Selevan, S. G., Hornung, R., Kissling, G. E., Cottrill, C., and Leffingwell, S. G. (1984). Reproductive Outcomes in Wives of Lead Exposed Workers, pp. 1-42. US National Institute for Occupational Safety and Health, Department of Health and Human Services, Cincinnati, OH.

Selevan, S. G., Rice, D. C., Hogan, K. A., Euling, S. Y., Pfahles-Hutchens, A., and Bethel, J. (2003). Blood lead concentration and delayed puberty in girls. *N Engl J Med* **348**, 1527-1536.

Shiau, C. Y., Wang, J. D., and Chen, P. C. (2004). Decreased fecundity among male lead workers. *Occup Environ Med* **61**, 915-923.

Shukla, R., Bornschein, R. L., Dietrich, K. N., Buncher, C. R., Berger, O. G., Hammond, P. B., and Succop, P. A. (1989). Fetal and infant lead exposure: effects on growth in stature. *Pediatrics* **84**, 604-612.

Shukla, R., Dietrich, K. N., Bornschein, R. L., Berger, O., and Hammond, P. B. (1991). Lead exposure and growth in the early preschool child: a follow-up report from the Cincinnati Lead Study. *Pediatrics* **88**, 886-892.

Siegel, M., Forsyth, B., Siegel, L., and Cullen, M. R. (1989). The effect of lead on thyroid function in children. *Environ Res* **49**, 190-196.

Silberstein, T., Saphier, O., Paz-Tal, O., Trimarchi, J. R., Gonzalez, L., and Keefe, D. L. (2006). Lead concentrates in ovarian follicle compromises pregnancy. *J Trace Elem Med Biol* **20**, 205-207.

Singh, B., Chandran, V., Bandhu, H. K., Mittal, B. R., Bhattacharya, A., Jindal, S. K., and Varma, S. (2000). Impact of lead exposure on pituitary-thyroid axis in humans. *Biometals* **13**, 187-192.

Slivkova, J., Popelkova, M., Massanyi, P., Toporcerova, S., Stawarz, R., Formicki, G., Lukac, N., Putala, A., and Guzik, M. (2009). Concentration of trace elements in human semen and relation to spermatozoa quality. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **44**, 370-375.

Sowers, M., Jannausch, M., Scholl, T., Li, W., Kemp, F. W., and Bogden, J. D. (2002). Blood lead concentrations and pregnancy outcomes. *Arch Environ Health* **57**, 489-495.

Srivastava, S., Mehrotra, P. K., Srivastava, S. P., Tandon, I., and Siddiqui, M. K. (2001). Blood lead and zinc in pregnant women and their offspring in intrauterine growth retardation cases. *J Anal Toxicol* **25**, 461-465.

Staessen, J. A., Nawrot, T., Hond, E. D., Thijs, L., Fagard, R., Hoppenbrouwers, K., Koppen, G., Nelen, V., Schoeters, G., Vanderschueren, D., Van Hecke, E., Verschaeve, L., Vlietinck, R., and Roels, H. A. (2001). Renal function, cytogenetic measurements, and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers. *Lancet* **357**, 1660-1669.

Stanek, K., Manton, W., Angle, C., Eskridge, K., Kuehneman, A., and Hanson, C. (1998). Lead consumption of 18- to 36-month-old children as determined from duplicate diet collections: nutrient intakes, blood lead levels, and effects on growth. *J Am Diet Assoc* **98**, 155-158.

Tang, N., and Zhu, Z. Q. (2003). Adverse reproductive effects in female workers of lead battery plants. *Int J Occup Med Environ Health* **16**, 359-361.

Telisman, S., Colak, B., Pizent, A., Jurasovic, J., and Cvitkovic, P. (2007). Reproductive toxicity of low-level lead exposure in men. *Environ Res* **105**, 256-266.

Telisman, S., Cvitkovic, P., Jurasovic, J., Pizent, A., Gavella, M., and Rocic, B. (2000). Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. *Environ Health Perspect* **108**, 45-53.

Tomoum, H. Y., Mostafa, G. A., Ismail, N. A., and Ahmed, S. M. (2010). Lead exposure and its association with pubertal development in school-age Egyptian children: pilot study. *Pediatr Int* **52**, 89-93.

Torres-Sanchez, L. E., Berkowitz, G., Lopez-Carrillo, L., Torres-Arreola, L., Rios, C., and Lopez-Cervantes, M. (1999). Intrauterine lead exposure and preterm birth. *Environ Res* **81**, 297-301.

U.S. EPA (2006). Air Quality Criteria for Lead. Office of Research and Development, National Center for Environmental Assessment, Washington, DC.

U.S. EPA (2007). Elevated Lead in D.C. Drinking Water - A study of Potential Causitive Events, Fional Summary Report, pp. 1-221. Office of Water, Washington, DC.

U.S. EPA (2011). Integrated Science Assessment for Lead (First External Review Draft). Office of Research and Development, National Center for Environmental Assessment-RTP Division, Research Triangle Park, NC.

Umeyama, T., Ishikawa, H., Takeshima, H., Yoshii, S., and Koiso, K. (1986). A comparative study of seminal trace elements in fertile and infertile men. *Fertil Steril* **46**, 494-499.

Vigeh, M., Yokoyama, K., Kitamura, F., Afshinrokh, M., Beygi, A., and Niroomanesh, S. (2010). Early pregnancy blood lead and spontaneous abortion. *Women Health* **50**, 756-766.

Vigeh, M., Yokoyama, K., Seyedaghamiri, Z., Shinohara, A., Matsukawa, T., Chiba, M., and Yunesian, M. (2011). Blood lead at currently acceptable levels may cause preterm labour. *Occup Environ Med* **68**, 231-234.

Vinceti, M., Rovesti, S., Bergomi, M., Calzolari, E., Candela, S., Campagna, A., Milan, M., and Vivoli, G. (2001). Risk of birth defects in a population exposed to environmental lead pollution. *Sci Total Environ* **278**, 23-30.

Viskum, S., Rabjerg, L., Jorgensen, P. J., and Grandjean, P. (1999). Improvement in semen quality associated with decreasing occupational lead exposure. *Am J Ind Med* **35**, 257-263.

Vivoli, G., Fantuzzi, G., Bergomi, M., Tonelli, E., Gatto, M. R., Zanetti, F., and Del Dot, M. (1993). Relationship between low lead exposure and somatic growth in adolescents. *J Expo Anal Environ Epidemiol* **3 Suppl 1**, 201-209.

Ward, N. I., Durrant, S., Sankey, R. J., Bound, J. P., and Bryce-Smith, D. (1990). Elemental Factors in Human Fetal Development. *Journal of Nutritional and Environmental Medicine* **1**, 19-26.

Ward, N. I., Watson, R., and Bryce-Smith, D. (1987). Placental element levels in relation to fetal developmentfor obstetrically "normal" births: a study of 37 elements. *International Journal of Biosocial Research* **9**, 63-81.

Wibberley, D. G., Khera, A. K., Edwards, J. H., and Rushton, D. I. (1977). Lead levels in human placentae from normal and malformed births. *J Med Genet* **14**, 339-345.

Williams, P. L., Sergeyev, O., Lee, M. M., Korrick, S. A., Burns, J. S., Humblet, O., DelPrato, J., Revich, B., and Hauser, R. (2010). Blood lead levels and delayed onset of puberty in a longitudinal study of Russian boys. *Pediatrics* **125**, e1088-1096.

Wolff, M. S., Britton, J. A., Boguski, L., Hochman, S., Maloney, N., Serra, N., Liu, Z., Berkowitz, G., Larson, S., and Forman, J. (2008). Environmental exposures and puberty in inner-city girls. *Environ Res* **107**, 393-400.

Wu, T., Buck, G. M., and Mendola, P. (2003). Blood lead levels and sexual maturation in U.S. girls: the Third National Health and Nutrition Examination Survey, 1988-1994. *Environ Health Perspect* **111**, 737-741.

Xu, B., Chia, S. E., Tsakok, M., and Ong, C. N. (1993). Trace elements in blood and seminal plasma and their relationship to sperm quality. *Reprod Toxicol* **7**, 613-618.

Xu, D. X., Shen, H. M., Zhu, Q. X., Chua, L., Wang, Q. N., Chia, S. E., and Ong, C. N. (2003). The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma. *Mutat Res* **534**, 155-163.

Yin, Y., Zhang, T., Dai, Y., Bao, Y., Chen, X., and Lu, X. (2008). The effect of plasma lead on anembryonic pregnancy. *Ann N Y Acad Sci* **1140**, 184-189.

Zailina, H., Junidah, R., Josephine, Y., and Jamal, H. H. (2008). The influence of low blood lead concentrations on the cognitive and physical development of primary school children in Malaysia. *Asia Pac J Public Health* **20**, 317-326.

Zentner, L. E., Rondo, P. H., and Mastroeni, S. S. (2006). Lead contamination and anthropometry of the newborn baby. *J Trop Pediatr* **52**, 369-371.

Zeyrek, D., Soran, M., Cakmak, A., Kocyigit, A., and Iscan, A. (2009). Serum copper and zinc levels in mothers and cord blood of their newborn infants with neural tube defects: a case-control study. *Indian Pediatr* **46**, 675-680.

Zhu, M., Fitzgerald, E. F., Gelberg, K. H., Lin, S., and Druschel, C. (2010). Maternal Low-Level Lead Exposure and Fetal Growth. *Environ Health Perspect* **118**, 1471-1475.

Zierler, S., Theodore, M., Cohen, A., and Rothman, K. J. (1988). Chemical quality of maternal drinking water and congenital heart disease. *Int J Epidemiol* **17**, 589-594.